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THE IDENTIFICATION AND EVALUATION OF TOXIC EFFECTS OF ORGANIC MICROPOLLUTANTS

by

JOHN W. SMITH, 1943

A DISSERTATION

Presented to the Faculty of the Graduate School of the

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ABSTRACT

THE IDENTIFICATION AND EVALUATION OF TOXIC EFFECTS OF ORGANIC MICROPOLLUTANTS

by John W. Smith

Advisor: Dr. Sotirios G. Grigoropoulos

1968

Rolla, Missouri

Organic micropollutants are found in both surface and subsurface water; their presence in water is considered highly objectionable because of the aesthetic problems, primarily color and taste and odor, which they impart to the water. However, of greater importance are the long-term effects of these materials. Because trace organics are not completely removed from surface waters by ordinary treatment processes and most subsurface waters are not treated in any manner, a serious health threat exists to the consumer by the presence of these materials.

This investigation was undertaken to characterize, identify, and evaluate the acute and long-term toxic effects of organic micropollutants in Missouri

waters. The development of a procedure for the prediction of long-term toxic levels from short-term studies and of new investigative techniques applicable to the trace organics field were of particular concern in this study.

Organic micropollutants were recovered from three subsurface waters using a modified carbon adsorption method consisting of three large (1.5 cubic foot) units in series and sequential elution with chloroform, alcohol, acetone, and benzene. A yearly composite of chloroform soluble organics recovered from treated river water with the standard PHS unit was also utilized. Significant concentrations of organics were found in spring and river water and detectable concentrations were present in well water.

Gas-liquid chromatographic and infrared analyses were employed in the identification studies. Gas-liquid chromatography was capable of separating most trace organics and collection of individual chromatogram peaks for post-chromatographic infrared analysis was possible. The use of gas-liquid chromatographic analysis demonstrated the complexity of the organics, but could not alone attain their identification. Partial identification was possible by evaluation of infrared spectra obtained by direct analysis and spectra of collected chromatogram peaks. The majority of the surface and subsurface water organic micropollutants were composed of homologous compounds containing aliphatic alcohol, aldehyde, and carboxylic acid functional groups and on the basis of their functional groups, their chemical characteristics, and probable origin, the organic micropollutants can be placed in the broad category of humic acids.

The acute and long-term toxicity of the trace organics was evaluated in batch-type bioassays. The carbon chloroform extract (CCE) and carbon

alcohol extract (CAE) materials recovered at the spring were not individually toxic to fish but in many cases exhibited strong synergistic behavior when combined at their naturally occurring ratio. The river water CCE and most spring water combined CCE and CAE materials were toxic to fish under both acute and long-term conditions. The surface water CCE was considerably more toxic than the most toxic subsurface water organics. Visual observations, respiratory enzyme studies using trout tissue homogenates in a Warburg respirometer, and oxygen transfer studies using a semipermeable membrane in a special test unit were used to evaluate the mode of action of the organics. Surface and subsurface water organic micropollutants were found to disrupt respiratory enzyme activity and physically clog the gills, respectively. A mathematical model was developed enabling estimation of toxic levels at any time and particularly the eventual median tolerance limit. It utilized a toxicity factor which normalized the effects of the toxicant mode of action and fish physical characteristics on the TLm value and employed a toxicity equation which had the form of

$$y_t = y_c + (y_o - y_c)\, e^{-bt}$$

where y_o and y_c are the immediate and eventual toxicity factors, y_t the toxicity factor at time t, and b a constant.

The organic micropollutants were very complex materials composed of homologous compounds and were toxic to fish under both acute and long-term conditions; the instrumentation and methodology employed to identify partially the trace organics and estimate their long-term toxicity can be applied equally well in treatment plant and research laboratories.

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Special thanks is also extended to those individuals who in some way assisted in this investigation.

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I. INTRODUCTION

Organic substances occur in the environment as the result of natural processes and are also added in large quantities by man in order to improve specific parts of the ecosystem and to dispose of waste products. Regardless of the source, many of these substances eventually end up in water supplies, subsurface and surface, where they are classified as organic micropollutants. Trace organics may, therefore, originate in water from several sources, including industrial and domestic wastes, accidental spillage, agricultural runoff, and bioresistant metabolic byproducts of the natural biota.

Today, the presence of trace organics in water is considered highly objectionable because of the aesthetic problems, primarily color and taste and odor, which they impart to the water. However, of greater importance than the aesthetic problems now recognized are the long-term toxic effects of these organic micropollutants. This is emphasized by the recovery of carcinogenic substances from drinking water in Japan (1) and West Germany (2) and the large scale fish kills in the lower Mississippi River due to the buildup of a pesticide in the fish (3).

In recognizing the aesthetic difficulties posed by the presence of the trace organics and the present inability to define clearly the chemical and toxicological nature of these materials, the U. S. Public Health Service has set the maximum permissible concentration of chloroform soluble organics in drinking water at 200 $\mu g/l$ (4, p. 31). While this is a step in the right direction, several investigators have found that in both surface (5, 6) and subsurface (7, 8) water the concentration of organics which are not chloroform

soluble is greater than the concentration of the chloroform soluble materials. The aesthetic (9,10) and toxicological effects of these materials cannot be overlooked. In addition, the synergistic or antagonistic relationships of the different organics in drinking water could be very great (10). Therefore, a need exists for additional knowledge on the nature and effects of these materials. This is even more imperative because trace organics are refractory; accordingly, their concentration is expected to increase with the increased water reuse resulting from the expanding population and economy. Because trace organics are not for the most part removed from surface waters by ordinary water treatment practices and subsurface waters are not usually treated in any manner, a serious health threat exists to the water consumer by the presence of these micropollutants. Consequently, it is the responsibility of the sanitary engineer to evaluate the toxicity of the organics and to develop methods for their destruction and/or removal from water.

The acute toxicity of the organic micropollutants to aquatic and other test animals can be evaluated in laboratory studies using existing procedures; however, the methodology required to establish the eventual or long-term toxic effects of these substances remains to be developed. Identification of the components of the trace organics is essential in order to evaluate fully the toxicological characteristics of these materials, especially with regard to man.

The ordinary characterization procedures employed in water analysis, including chemical and biochemical oxygen demand, taste and odor, and solubility partitioning, reveal very little of the identity of the organic micropollutants; sophisticated equipment and procedures must be employed to identify

the organics. Various forms of chromatography (paper, column, thin-layer, and gas-liquid) and spectroscopy (infrared, ultraviolet, and fluorescent) have been employed by different investigators to study trace organics. Of these techniques, only fluorescence spectral analysis has been utilized in specific identification studies (2) to determine carcinogenic materials present in drinking water.

Identification of the components of the organic micropollutants would also assist in the development of new methods for their destruction and/or removal. The majority of the presently employed water and waste water treatment processes have been shown inefficient in removing trace organics from water (9, 11, 12, 13). The use of activated carbon is the only process which has been found applicable (11, 14); however, this method is not being used to its fullest capabilities. Equipped with the necessary methodology and instrumentation, water and waste treatment plant laboratories could perform inplant identification and characterization studies which would be of material aid in applying appropriate treatment procedures to remove these pollutants and produce a safe water of an improved aesthetic quality.

This investigation is part of a research study whose scope is the recovery of organic micropollutants from surface and subsurface Missouri waters; the characterization and identification of these substances; the evaluation of their toxic effects, both acute and long-term; and the development of methods for their destruction and/or removal. The author has previously reported on the recovery and general characterization of organic micropollutants from three Missouri subsurface waters in a Master's thesis (10).

The overall objectives of the present investigation were the evaluation of the acute and long-term toxic effects and the identification of organic micropollutants from Missouri surface and subsurface waters. The specific objectives of this study were (a) the evaluation of the acute and long-term toxic effects of the trace organics, (b) the development of a procedure for the prediction of long-term toxicity on the basis of short-term studies, (c) the development of the methodology necessary for the identification of the organics; and (d) the evaluation of the identification procedures with selected organic micropollutants.

Materials used in this study included chloroform, alcohol, acetone, and benzene soluble organics recovered from three Missouri subsurface waters and a yearly composite of chloroform soluble organics from treated Missouri River water. The acute and long-term toxicity of these substances was determined in batch-type bioassays. The mode of action of the trace organics was evaluated with respiratory enzyme and oxygen transfer studies and subsequently utilized in coordination with the toxicity findings to develop a procedure for predicting long-term toxic levels. Gas-liquid chromatography and infrared spectroscopy were evaluated individually and in combination as practical identification techniques.

In summary, it was the purpose of this investigation to characterize, identify, and determine the toxic effects of organic micropollutants in water. In addition to the direct contribution to the present-day knowledge in the field of trace organics, this investigation should also make a significant contribution through the development of new investigative techniques applicable to this field.

The procedure enabling the evaluation of long-term toxic effects on the basis of short-term data will serve both research investigators and those individuals who are responsible for the abatement of pollution and the production of a palatable and safe water. The methodology developed for the identification of organic micropollutants would be of considerable value to research laboratories in the water and waste water field, while at the same time the required instrumentation is not out of reach of the laboratories of the larger treatment plants. In fact, the increased severity of the aesthetic problems caused by the presence of organic micropollutants and the increased interest in the toxicological characteristics of these organics make it necessary for the water and waste water treatment industry to adapt and employ identification techniques in order to assure the production of a high quality water and the protection of streams and other natural water bodies.

II. REVIEW OF LITERATURE

The purpose of this literature review is to present investigations pertinent to the recovery and general characterization of organic micropollutants from water, the evaluation of their toxic effects, and the identification of their components.

A. RECOVERY AND GENERAL CHARACTERIZATION OF ORGANIC MICROPOLLUTANTS

Organic micropollutants are found in water in trace amounts; consequently, a means of concentrating and recovering these organics from water must be utilized before characterization studies can be undertaken. Investigations pertaining to the recovery and general characterization of these substances are reported in this chapter.

1. Recovery

The method of recovery which has been most widely used in the United States is the carbon adsorption method (CAM) developed in the early 1950's by Braus, et al. (15) at the Robert A. Taft Sanitary Engineering Center. The CAM involves passing a known volume of water through an 18 inch high, 3 inch diameter column packed with activated carbon, and serially extracting the dried carbon with chloroform to desorb the organics. This method has been accepted as a standard procedure (16, p. 215) for the determination of organic compounds in water and is used in conjunction with the 200 μg/l limit that has been set for chloroform soluble organics in drinking water (4, p. 31).

The procedure developed by Braus, et al. (15) has been modified by several investigators to accomplish different experimental goals. These

modifications, summarized in Table 1, range from an alteration of the pretreatment facility to the utilization of several solvents to elute the dried carbon.

The necessity of more than one filter in series was investigated by Greenberg, et al. (19) in studies undertaken to evaluate the efficiency of the CAM. They reported that organic materials were present in the effluent of the PHS filter and concluded that a second filter in series should be employed for effective recovery of the trace organics. Grigoropoulos and Smith (7), who investigated the presence of trace organics in subsurface waters, reported that in some cases three large capacity activated carbon filters were not effective in recovering all organics and concluded that a fourth unit was needed. In both investigations (7,19), the material recovered on the second and third filter could have been physically washed from the first filter because of overloading or selectively adsorbed on the second or third filter because of its characteristics. In either case, it is evident that for effective and representative recovery of organics from surface and subsurface waters at least two filters in series are required.

The size of the PHS filter severely limits the quantity of organics which can be recovered on one filter. Consequently, several investigators (5, 7, 9, 18) have employed large capacity activated carbon filters containing up to 20 times the volume of the standard filter in order to recover enough material for characterization and identification studies. Using two large capacity filters in

Table 1

Modifications of the Carbon Absorption Method

Investigators	Ref.	Source of Water	Modification	Procedure Utilized
Braus, et al.	15	Cincinnati River	Standard filter	Sand filtration; PHS (0.073 cubic foot) filter; elution with chloroform.
Middleton, et al.	17	Cincinnati River	Pretreatment	Sedimentation and sand filtration; PHS filter; elution with chloroform.
Middleton, et al.	18	Cincinnati River	Pretreatment; size and number of filters; elutants used.	Sedimentation and sand filtration; two large capacity (1.3 cubic foot) filters in series; elution with chloroform and ethanol.
Myrick and Ryckman Dornbush and Ryckman Spicher and Skrinde	9 11 12	Missouri River	Pretreatment; size and number of filters; pH of water; elutants used.	Sedimentation and diatomite filtration; two large capacity (1.2 cubic foot) filters in series; pH adjustment to 2.0 before second filter; elution with chloroform, ethanol, acetone, and benzene.
Grigoropoulos and Smith	7	Missouri spring and deep wells	No pretreatment; size and number of filters; elutants used.	Three large capacity (1.5 cubic foot) filters in series; elution with chloroform, ethanol, acetone, and benzene.
Robinson, et al.	8	Illinois wells	No pretreatment; number of filters; pH of water; elutants used; pH of elutants.	Three PHS filters in series; pH adjustment to pH 2.0; elution with chloroform, ethanol, ethanol plus ammonia, and ethanol plus hydrochloric acid.

series, the Taft Center group (5,18) recovered 1600 grams of CCE* from Cincinnati River water, while Myrick and Ryckman (9) obtained 27.7 and 85.6 grams of CCE and CAE,* respectively, from Missouri River water; using three large capacity filters in series, Smith (10) obtained 46 and 98 grams of CCE and CAE, respectively, from a Missouri subsurface water.

Acidification of the effluent from the first filter before it was charged onto the second filter in the series has been employed by several investigators (8,9,11,12) in order to improve the efficiency of recovery both qualitatively and quantitatively. The procedures utilized are described in Table 1, and their findings are summarized below:

		Recovery, % of Unit #1			
		Unit #2		Unit #3	
Investigators	Ref.	CCE	CAE	CCE	CAE
Washington University Group	9,11,12	80	170	--	--
Robinson, et al.	8	100	200	50	100

It should be noted, however, that Grigoropoulos and Smith (7) have concluded on the basis of the recoveries of organic micropollutants from subsurface water obtained with the second and third filter without pH adjustment that possibly part of the organics recovered on the second and third filters using acidified water may have been recovered without acidification.

---

*CCE: Carbon Chloroform Extract; CAE: Carbon Alcohol Extract.

Although chloroform had been used originally as the elutant for the CAM (15), several investigators (5, 6, 7, 8, 9) have employed additional solvents to recover those trace organics which were not chloroform soluble. Myrick and Ryckman (9) have reported the recovery from Missouri River water of 317, 105, and 84 μg/l ethanol, acetone, and benzene soluble organics, respectively, in addition to 81 μg/l of chloroform soluble materials. Grigoropoulos and Smith (7) also have reported the recovery of 109, 71.5, and 31.7 μg/l ethanol, acetone, and benzene soluble organics, respectively, in addition to 43.7 μg/l of chloroform soluble materials from a Missouri subsurface water.

The effect of pretreatment, as well as acidification and elution with various solvents, on the recovery efficiency of the PHS filter was investigated by Rock, et al. (20) using a mobile microcontaminant collector in studies undertaken to determine the effect of turbidity on the CAM. Surface water from the Mississippi River and the Huzzah River, a relatively unpolluted stream in a Missouri forest area, was pretreated with diatomatecous earth, sand, or fiberglass and passed through two PHS filters in series, with the second filter receiving acidified water (pH 2.5). Parallel studies were performed using two PHS filters but without acidification. The carbon was sequentially eluted with chloroform, ethanol, acetone, and benzene. The authors presented the results of their studies in bar-graph form and reported that while greater quantities of organics were recovered under acidic conditions when the turbidity was removed, turbidity did not exert a significant effect on the recovery at the natural pH. They reported further that the first filter was overloaded, as evidenced by

equal or greater recoveries on the second filter at natural pH, and that either the volume of water sampled or the filtration rate should have been reduced.

Several other methods have also been proposed for the recovery of organic materials from water, including liquid-liquid extraction, freeze-drying, and the use of ion-exchange columns. Liquid-liquid extraction has been employed by Morris, et al. (21) and Lure and Ponova (22) to concentrate organic materials from water and to identify specific organic compounds by color comparison with known standards. The method as used by these investigators did not furnish an organic extract which could be subjected to further study. Other investigators (23, 24, 25, 26), however, have employed liquid-liquid extraction to recover organic materials from water which were then subjected to various forms of identification (gas-liquid and thin-layer chromatography, and elemental chemical analysis). Freeze-drying has been used by Baker (27) and Sugar and Conway (28) to recover organic micropollutants from water which were then subjected to gas-liquid chromatographic analysis and by Midwood and Felbeck (29) who analyzed the organics by infrared spectrophotometric techniques. Ion-exchange columns have been employed by several investigators (30, 31, 32, 33) to remove color causing compounds from water. Large volumes of water can be sampled with an ion-exchange column which would enable a sufficient amount of organic material to be recovered for characterization and identification studies.

The various methods for the recovery of organic micropollutants from water are summarized in Table 2. Each procedure has certain advantages and disadvantages, and for each procedure there is a particular application for which

Table 2

Methods for the Recovery of Organic Micropollutants

Method	Application	Determinations Possible With Recovered Material
Carbon Adsorption	Monitoring and concentrating device; large volume of water can be sampled and sufficient material can be recovered for extensive characterization and identification studies; any solvent may be used; several days required for results.	General characterization, solubility partitioning, toxicity studies, chromatographic and spectrometric studies, identification, organoleptic studies.
Liquid-Liquid Extraction	Concentrating device; limited volume of water can be sampled with small amount of extract recovered, therefore, identification by sophisticated equipment only; solvent must be immiscible with water; several hours required for results.	Chromatographic and spectrometric identification.
Freeze-Drying	Concentrating device; limited volume of water can be sampled with small amount of extract recovered, therefore, identification by sophisticated equipment only; solvent, when used, must be immiscible with water; several hours required for results.	Chromatographic and spectrometric identification.
Ion-Exchange	Monitoring and concentrating device; large volume of water can be sampled and sufficient material can be recovered for extensive characterization and identification studies; solvent must be immiscible with water; several days required for results.	General characterization, solubility partitioning, toxicity studies, chromatographic and spectrometric studies, identification, organoleptic studies.

it is best suited. Liquid-liquid extraction and freeze-drying can be used to recover organic materials from water in a relatively short period of time; however, the volume of sample and solvents which can be used are limited. These practical limitations require that sophisticated equipment be employed for the analysis of the extract and restrict the number of studies which may be performed on the extract. The carbon adsorption and ion-exchange methods, though less efficient than liquid-liquid extraction and requiring many days instead of several hours, allow the sampling of a large volume of water thus providing a large quantity of sample for evaluation. Sophisticated equipment is required for the identification of the materials obtained, but characterization and toxicity studies may be performed to evaluate further the organics. The ion-exchange method is limited by the requirement of an immiscible solvent; however, the CAM is not limited by the type of solvent and, consequently, compounds which are soluble in a solvent miscible with water may be recovered and evaluated. While all the recovery methods listed in Table 2 are affected by turbidity, liquid-liquid extraction and freeze-drying are perhaps affected to a greater extent than are the CAM and ion-exchange method which can be employed in conjunction with a pretreatment facility.

2. Characterization of Organic Micropollutants

The characterization of organic micropollutants involves several techniques. Perhaps the method most widely used is the solubility partitioning of the organic extracts (34, 35), which separates the organic materials into different groups (Table 3) on the basis of their solubility in a series of solvents. Myrick and Ryckman (9) utilized solubility partitioning to characterize several

Table 3

Solubility Partitioning of Organic Micropollutants

Test Material	Fraction, %												Ref.
	Ether Insolubles	Water Solubles	Weak Acids	Strong Acids	Bases	Amphoterics	Neutrals					Others	
							Total	Aliphatics*	Aromatics*	Oxygenated*	Others*		
Missouri River													
CCE	9.5	19.5	12.8	11.5	1.00	2.80	34.2	3.2	7.2	87.8	1.8	8.6	9
CAE	>99.0	--	--	--	--	--	--	--	--	--	--	--	
Missouri River Composite CCE	2.2	25.8	10.0	6.3	1.70	--	26.3	11.7	6.9	76.5	4.9	27.7	13
Meramec Spring Run #1 Unit #1													
CCE	10.0	31.0	8.5	11.8	0.77	0.91	11.8	16.1	36.9	37.0	10.0	25.2	
CAE	>99.0	--	--	--	--	--	--	--	--	--	--	--	
Unit #2													
CCE	5.0	42.0	7.4	12.8	0.40	0.60	14.2	4.2	45.5	41.7	8.6	17.6	
Unit #3													
CCE	6.9	13.5	14.0	7.0	0.00	0.00	48.0	50.8	5.0	25.4	18.8	10.6	
CAE	44.0	33.0	2.5	9.5	0.20	0.20	3.1	16.2	0.0	56.7	27.1	7.5	
Meramec Spring Run #2 Unit #1													7
CCE	2.1	27.0	13.6	13.4	0.83	0.35	14.0	22.0	24.5	50.5	3.0	28.7	
CAE	>99.0	--	--	--	--	--	--	--	--	--	--	--	
Unit #2													
CCE	1.2	30.0	9.6	10.6	1.00	0.90	23.0	6.8	21.0	44.3	27.9	23.7	
UMR Well													
Unit #1													
CCE	6.4	15.0	6.8	11.5	1.50	1.60	21.4	58.5	19.6	21.6	10.3	35.8	
CAE	>99.0	--	--	--	--	--	--	--	--	--	--	--	
CAcE-1**	1.6	5.0	8.3	5.0	0.60	0.50	71.0	0.0	77.7	20.9	1.0	8.0	
CBE-2**	0.0	0.6	2.2	0.6	0.50	0.20	83.5	5.0	56.2	25.4	13.4	12.4	

*% of total neutral fraction.

**The numbers 1 and 2 refer to the order in which acetone and benzene were employed.

CCE samples recovered from Missouri River water at St. Louis (see Table 1, p. 8), and Grigoropoulos and Ryckman (13) employed the method for the group breakdown of a composite CCE sample prepared from extracts obtained over a period of one year from Missouri River water at St. Louis using a PHS filter. Grigoropoulos and Smith (7) used this separation technique to characterize CCE and CAE samples recovered in two separate runs from the water of Meramec Spring in Missouri, and CCE, CAE, CAcE*, and CBE* samples obtained from a deep well water located at the University of Missouri - Rolla (UMR) campus (see Table 1, p. 8). The results of these studies (7, 9, 13) are summarized in Table 3. Myrick and Ryckman (9) also attempted to characterize the CAE from Missouri River water, but found that the extracts were 99 per cent ether insoluble. Grigoropoulos and Smith (7) reported similar results for the CAE recovered with the first unit of each filter run using subsurface water but found that an alcohol extract of a second unit was ether soluble and could be separated by the partitioning procedure. These results are included in Table 3.

Ryckman, et al. (36) sampled Missouri River water at eight points along an 800 mile stretch of the river using eight PHS filters and utilized solubility partitioning to characterize the CCE recovered at the various points. The authors reported that while the concentration of the ether insoluble and amine (basic) organics remained constant over the 800 mile sampling region, all the other solubility fractions exhibited significant increases at the downstream sampling points.

---

*CAcE: Carbon Acetone Extract; CBE: Carbon Benzene Extract.

Another technique which has been employed in the characterization of organic micropollutants is elemental chemical analysis accompanied by the development of empirical formulas. Results obtained in several previously discussed investigations (7, 8, 9, 13) are summarized in Table 4. Also presented in this table are the results of the microchemical analysis of an organic extract recovered by Goncharova and Datska (25) from a European well water by liquid-liquid extraction.

The oxygen demand of the organics, both chemical and biochemical, has also been determined in many of these investigations (7, 8, 9) and the results reported are presented in Table 5. Also presented in this table are the data obtained by Ryckman, et al. (36) and Tengonciang (37) for trace organics recovered at the eight sampling points along the 800 mile stretch of the Missouri River.

Although the general characteristics determined by these procedures are satisfactory in most cases for comparative purposes, they reveal very little of the identity of the components of the trace organics. Therefore, the use of sophisticated techniques (reported in Section C of this chapter) not normally utilized by the sanitary engineer is necessary to provide more information on the identity of the organic micropollutants.

B. TOXICITY

Although many of the difficulties caused by the presence of organic micropollutants in water (taste and odor, color, acute toxicity, fouling of ion-exchange columns, and foaming) have been established, relatively little is known of the long-term effects of these materials. The potential health threat

Elemental Composition and Empirical Formulas of Organic Micropollutants

Test Material	Elemental Composition, %						Empirical Formula	Ref.
	C	H	O	N	S	P		
Missouri River								
CCE	66.0	7.10	19.2	0.84	1.60	--	$C_{5.5}H_{7.1}O_{1.2}N_{0.06}S_{0.05}$	
CAE	30.0	6.10	33.6	2.38	0.96	--	$C_{2.5}H_{6.1}O_{2.1}N_{0.17}S_{0.03}$	9
Missouri River Composite CCE	65.6	8.40	25.1	1.40	0.70	None	$(C_7H_{11}O_2)_x$	13
Meramec Spring Run #1 Unit #1								
CCE	59.0	6.96	24.2	0.68	<0.10	0.36	$C_{4.5}H_{6.8}O_{1.5}N_{0.05}P_{0.01}$	
CAE	51.2	6.52	36.0	2.53	<0.10	0.35	$C_{4.2}H_{6.4}O_{2.4}N_{0.18}P_{0.01}$	
Meramec Spring Run #2 Unit #2								
CCE	62.5	7.40	24.0	0.54	0.20	0.22	$C_{5.2}H_{7.4}O_{1.5}N_{0.04}P_{0.01}S_{0.01}$	7
CAE	46.3	7.47	36.6	2.04	0.13	2.80	$C_{3.9}H_{7.5}O_{2.3}N_{0.14}P_{0.09}$	
UMR Well Unit #1								
CCE	52.5	6.74	14.7	0.32	27.80	0.22	$C_{4.2}H_{6.4}O_{0.92}N_{0.02}P_{0.01}S_{1.0}$	
CAE	42.9	6.81	36.0	1.36	<0.10	0.24	$C_{3.6}H_{6.8}O_{2.3}N_{0.10}P_{0.01}$	
Illinois Ground Water								
CCE	57.8	7.00	30.1	--	--	--	$C_{4.8}H_{7.0}O_{1.9}$*	
CAE	45.4	5.20	46.4	--	--	--	$C_{3.8}H_{5.2}O_{2.9}$*	8
Well Water Liquid-Liquid Extract	42.8	7.00	39.8	3.22	--	--	$C_{4.4}H_{7.0}O_{2.3}N_{0.23}$	26

*Empirical formula was not reported but was developed on the basis of the elemental composition given.

Table 5

Oxygen Demand of Organic Micropollutants

Test Material	Chemical		5-Day Biochemical		Ref.
	mg O_2/mg extract	% Theoretical	mg O_2/mg extract	% COD	
Missouri River CCE					
at Yankton	2.14	--	0.07	3.3	
Omaha	2.15	--	0.06	2.8	
St. Joseph	2.20	--	0.22	10.0	
Kansas City	2.20	--	0.25	11.6	36, 37
Lexington	2.11	--	0.09	4.3	
Jefferson City	2.01	--	0.04	2.0	
St. Louis City	2.35	--	0.06	2.6	
St. Louis County	2.25	--	0.06	2.7	
Missouri River					
Unit #1					
CCE	2.31	--	--	--	
CAE	1.14	--	--	--	
Unit #2*					9
CCE	1.52	--	--	--	
CAE	1.10	--	--	--	
Meramec Spring					
Run #1 Unit #1					
CCE	1.37	74.0	0.26	19.3	
CAE	1.49	98.0	0.23	15.3	
Unit #2					
CCE	1.48	--	0.13	8.7	
CAE	1.30	--	0.10	8.0	
Unit #3					
CCE	1.35	--	0.15	11.1	
CAE	1.40	--	0.14	10.0	
Meramec Spring					
Run #2 Unit #1					
CCE	1.32	--	0.18	12.9	7
CAE	1.31	--	0.22	16.5	
Unit #2					
CCE	2.00	99.5	0.16	8.2	
CAE	1.40	94.5	0.19	13.6	
Unit #3					
CCE	1.72	--	0.14	8.4	
CAE	1.94	--	0.13	6.7	
UMR Well					
Unit #1					
CCE	1.64	95.3	0.00	--	
CAE	1.46	94.2	0.00	--	
Illinois Ground Water					
Unit #1					
CCE	1.67	84.0	--	--	
CAE	1.23	--	--	--	8
Unit #2*					
CAE	1.27	80.5	--	--	

*Water was acidified to pH 2.0 before it was passed through the unit.

posed by these organics is emphasized by the identification of specific carcinogenic agents in drinking water in West Germany by Borneff, et al. (38, 39, 40, 41, 42) and in Japan by Takemura, et al. (1).

Borneff and his coworkers have recovered and identified the known carcinogen 3,4-benzopyrene and other fluorescent polycyclic aromatic compounds from river water. Borneff (41) also evaluated the synergistic or antagonistic relationship between the carcinogen and nonbiodegradable detergents in river water by means of feeding experiments with mice using 3,4-benzopyrene and ABS. Carcinomas were produced by the benzopyrene only when it was solubilized by the detergent in drinking water. When benzopyrene was fed in the dry food, the presence or absence of the detergent in the drinking water did not have an effect on the frequency of carcinoma production by benzopyrene. The author concluded that drinking water obtained from river water contaminated with both bioresistant detergents and benzopyrene compounds must be considered injurious to health. Borneff, et al. (42) further evaluated syncarcinogenic effects of chromate and 3,4-benzopyrene on mice. The toxicants were fed to three generations of mice in their drinking water at an average concentration of 10 μg/ml (average daily consumption of 18 μg) benzopyrene and 500 μg/ml (average daily consumption of 900 μg) chromate. In over two years of experimentation, the frequency of benzopyrene-induced gastric tumors was not increased by the presence of chromate.

Takemura, et al. (1) recovered and identified aromatic amines with known carcinogenic properties from river water in Japan. They reported that the river received untreated industrial wastes from dye manufacturing industries

but had no known sources of carcinogenic amines. In laboratory studies Takemura and his associates demonstrated that the azo dyes in the untreated effluents could be reduced to aromatic amines by hydrogen sulfide or sulfur dioxide in the river water. To alleviate the danger involved, they strongly recommended that waste treatment facilities be installed at the offending dye factories.

Hueper and Payne (43) evaluated the long-term physiological impact of 2,4-benzopyrene and 1,2-benzoanthracene on mice using a direct injection technique. The adsorbates were thought to be the probable cause of leukemia in some of the test animals and the cause of carcinoma at the point of injection and the bladder of other mice.

Weiss and Gakstatter (44) studied the toxicity of organophosphorus pesticides to fish brain cholinesterase activity. Fish brain tissue was removed from healthy fish, homogenized, and exposed to different concentrations of pesticides at different pH levels. The inhibition of cholinesterase activity was determined by comparing the enzyme activity of the test solutions with the activity of a control solution which did not contain any pesticide. Weiss and Gakstatter also reported the results of long-term bioassays with trout exposed to low concentrations (0.01 mg/l) of the organophosphorus pesticides. Fish were periodically removed from the bioassay test solutions, and the brain cholinesterase activity was determined. They found that the enzyme activity was reduced to about 50 per cent of normal activity in 10 days, at which time an equilibrium was reached and no further decrease was noted.

The technique of using known compounds to study adverse effects is one way of evaluating the toxicity of organic micropollutants if the identity of the organics is known. Unfortunately, the complete evaluation of the toxicity of trace organics cannot be based on the results obtained using one or two identified compounds. This procedure would be very useful if all the components were identified and the synergistic and/or antagonistic effects of the various materials were evaluated. To overcome this difficulty, some investigators have attempted to determine in toto the toxicity of complex organics removed from water or waste water. Sproul and Ryckman (45) at Washington University in St. Louis have evaluated the acute toxic effects to trout of trace organics recovered from industrial wastes, domestic wastes, and river water using an activated carbon filter and elution with chloroform and ethanol. The extracts exhibited a wide range of toxicities to the test fish depending on the source of the organic micropollutants. The organics from a chemical waste were the most toxic (all test fish were killed within 3 hours at 5 mg/l), while the extracts from domestic sewage and river water were the least toxic (no deaths occurred at 100 mg/l in a period of 6 days). Although fish, instead of humans, were employed in this study, the detrimental effects found demonstrated the need for further evaluation and consideration.

Sletten (46), also at Washington University, evaluated the physiological response of fish and fish tissue homogenates to organic micropollutants found in St. Louis tap water. The organics were recovered from the water using two large capacity carbon filters in series with the second filter receiving acidified water. Elution of the carbon was with chloroform and ethanol. Acute

bioassay studies were performed with the CCE and CAE using fingerling rainbow trout as test fish. The chloroform soluble materials were toxic to the fish at low concentrations (96 hour median tolerance limit of 28 mg/l), while the CAE had no effect on trout exposed to 100 mg/l for 5 days. The synergistic effects of the CCE and CAE were not studied; however, they could have been significant. In an attempt to evaluate better the toxicity of the organics and to determine the probable mode of action, enzyme studies were performed using homogenized trout liver, heart, and gill tissue in a Warburg respirometer. When exposed to the CCE, the homogenates exhibited a definite inhibition of respiratory enzyme activity, but showed no inhibition when they were exposed to the CAE; again the combined effects of the two materials were not considered. It would seem reasonable to expect on the basis of the results obtained by Sletten (46) that in addition to acute toxicity these organics could exhibit significant long-term toxic effects as emphasized by the inhibition of respiratory enzyme activity.

The measurement of long-term toxic effects of a material requires either extended test periods or a suitable procedure for estimating future toxicity levels from short-term studies. Abram (47,48) has evaluated several methods of determining long-term toxic levels of pesticides on rainbow trout and harlequin fish. Both continuous flow-constant concentration and continuous flow-fluctuating concentration operations were utilized in long-term studies of up to three months duration and in short-term studies used to predict long-term levels mathematically. Abram employed the harmonic mean survival time in his experiments conducted at a constant concentration and varying length of time, rather than the

usual American practice of a constant time and varying concentration which leads to a median tolerance limit. He defined the harmonic mean survival time (which is close to the median time) as the time required to kill 50 per cent of a batch of fish as determined by harmonic rather than linear relationships. Assuming that three months represented an infinite time for the test fish, he concluded that it was possible to predict the long-term values arrived at experimentally on the basis of short-term studies. Abram also found that the harmonic mean survival times of fish exposed to fluctuating concentrations and fish exposed to a constant concentration were approximately the same and concluded that although the concentration of a toxicant may fluctuate around the threshold level (which is just on the borderline of toxicity), under natural conditions the fish would behave as if exposed to the average concentration of the fluctuations. He further stated that the difference between the concentrations which would kill all or none of the test fish under prolonged exposure was very small.

C. IDENTIFICATION OF ORGANIC MICROPOLLUTANTS

The organic micropollutants extracted from aqueous solutions consist of such numerous and varied chemical compounds as to make their separation and identification an extremely difficult process requiring the most refined and sophisticated methods available. The two analytical techniques which have been employed with the greatest success to date are chromatography and spectroscopy. However, even with these techniques which have a great potential for identification, prior separation of the materials into simpler compounds is often necessary.

Chromatography (paper, column, thin-layer, and gas-liquid) has been utilized as both a separation and an identification technique in the analysis of trace organics. Paper and column chromatography have primarily been employed as separation procedures with the fractions analyzed by other chromatographic or spectrophotometric methods. Goren-stul, et al. (49) used paper chromatography to separate various phenolic compounds recovered from river water with the carbon adsorption method (PHS filter). The CCE was first broken down into various fractions by solubility partitioning, and the fractions were further separated using paper chromatography. The paper chromatograms were developed with butanol and benzene saturated water and molybdophosphoric acid as a spraying agent. The compounds separated with paper chromatography were eluted from the paper with chloroform and analyzed with a gas chromatograph using a 1.5 meter (4.5 foot) long, 4 mm (1.6 inch) diameter column with 10 per cent tris (2,4-xylenyl) phosphate on 60/80 mesh Chromosorb W. Gorenstul, et al. (49) were able to identify 18 phenols and chlorophenols in the CCE. They also attempted to use direct infrared analysis of the fractions obtained by solubility partitioning, but concluded that due to the presence of isomers in the fractions, infrared analysis was inefficient without further separation.

The routine identification of pesticides in Missouri River water at Omaha, Nebraska, has been reported by Goodenkauf and Erdei (50). The procedure employed by these investigators consisted of recovery of the pesticides in a CCE using a PHS filter, solubility separation of the CCE, column chromatographic separation of the neutral fraction, and paper chromatographic separation of the aromatic group from the neutral fraction. The pesticides were then

identified by developing the paper chromatogram with a silver nitrate chromogenic agent and comparing the unknown compounds with known standards.

Lindgren (51) has utilized a combination of liquid-liquid extraction, column chromatographic separation, and infrared analysis to study quantitatively the amount of petroleum residues in lake water. Several liters of water were extracted with one-half liter of carbon tetrachloride, the solvent was evaporated leaving a residue, and the residue was fractionated with column chromatography. The fractions were analyzed at wavelengths of 3.38, 3.42, and 3.51 microns to detect the petroleum residues. Lindgren was able to confirm the presence of gasoline and motor oil residues in the water and reported that the procedure was sensitive to concentrations as low as 50 μg/l.

Smith and Eichelberger (24) have employed thin-layer chromatography to identify several pesticides present in a CCE and an aromatic fraction. Carbon tetrachloride was used as the developing agent, and the separated components were located using Rhodamine B spray reagent and ultraviolet light. Gas-liquid chromatography using a microcoulometric titration detector was employed to identify positively lindane, aldrin, heptachlor, dieldrin, and DDT.

Although the identification of certain organic materials has been accomplished using paper chromatography, many investigators are now turning to gas-liquid chromatography because of its sensitivity (detectable concentrations in the picogram* range) and its enormous separating capabilities. However,

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*One picogram equals 10^{-12} grams.

even with gas-liquid chromatographic analysis, prior separation of the sample into its components and clean-up procedures are required.

The applicability of gas-liquid chromatographic analysis to industrial wastes was investigated by Sproul, et al. (52). The carbon adsorption method was employed to recover organic micropollutants from various types of industrial wastes, domestic wastes, and river water. The recovered CCE and CAE samples were analyzed with a gas chromatograph. Toluene and orthodichlorobenzene were identified in the CCE from a chemical plant waste, while various fatty acids from caprylic to linolenic were thought to be in the CCE and CAE from brewery, corn refining, meat packing, paint and soap industrial wastes, and domestic wastes. Although Sproul and his coworkers did not utilize a clean-up or separation technique before the gas-liquid chromatographic analysis, they reported that each extract was composed of many compounds (29 in one extract) and that identification of the reported materials was greatly facilitated by prior knowledge of the composition of the wastes.

Lamar and Goerlitz (23) studied liquid-liquid extraction and vacuum evaporation as concentrating methods prior to gas-liquid chromatographic analysis aiming at the identification of carboxylic acids in unpolluted surface streams. The carboxylic acids were recovered from a 23.5 liter volume of surface water by continuous liquid-liquid extraction with n-butanol, extraction of the n-butanol with a 5 per cent solution of sodium bicarbonate, acidification of the aqueous layer, and reextraction with n-butanol. The vacuum evaporation process consisted of reducing a 23.5 liter volume of water to 150 ml and extracting with a diethyl ether-ethanol mixture to remove unwanted neutral and basic materials.

The aqueous layer was acidified and steam-distilled to isolate the carboxylic acids. The acids recovered by each procedure were subjected to gas-liquid chromatographic analysis using both polar and non-polar columns, and the retention volumes compared with standards for identification. Twelve carboxylic acids were identified and eight more were thought to be present but were not identified. The authors (23) concluded that regardless of the recovery technique utilized changes in the specific character of the organics can be expected to occur during the recovery procedure. Six low molecular weight fatty acids were also identified using gas-liquid chromatography in an organic material recovered with an ion-exchange column from lake water in Soviet Russia (33).

Baker (53) recently presented a summary of some of the practical operating considerations and recent developments in measuring trace organics in aqueous solution. Because of the changes which may occur during concentration and recovery, Baker advocated direct aqueous injection into a gas chromatograph for identification of organic micropollutants, but conceded that the concentration of organics should be in the mg/l range for good results. In laboratory studies, he found that lower concentrations of known materials were often difficult to detect. It should be pointed out that a concentration of at least 10 to 20 fold is required with most surface waters, and even greater with many subsurface waters, before mg/l quantities of organic micropollutants can be obtained.

Gas chromatography in combination with liquid-liquid extraction has been employed by Caruso, et al. (25) to trace sources of taste and odor causing organic micropollutants and study the variations in concentration of organics in a lake and a river water. The water was extracted with ether, the ether layer

was separated, concentrated, and a 2 μl volume of the concentrated ether solution of organics was injected into the gas chromatograph to form a "fingerprint" of the organics present. By comparing fingerprints of extracts from different points along the stream and the lake shore, the authors (25) concluded that the organics found in the river water were contributed by surface runoff and domestic sewage rather than by industrial effluents. The trace organics found in the lake water were present at lower concentrations than those in the river water but exhibited similar fingerprints on the gas chromatograph.

Other investigators (54, 55, 56) have employed gas-liquid chromatographic techniques in the analysis of river water for pesticides. May, et al. (54) utilized carbon adsorption followed by benzene elution to recover pesticides from river water and subjected the organics thus obtained to both paper and gas-liquid chromatographic analysis. They reported that although paper chromatography was less expensive than gas chromatography in terms of equipment and was easier to perform, the method was not quantitative, it required a very efficient clean-up procedure preceding analysis, and was affected by the presence of isomers. Consequently, May and his coworkers utilized gas-liquid chromatography to identify aldrin, DDT, and 2,4-D in the benzene extract. They reported that the microcoulometric titration detector gave better results than either the flame ionization or electron capture detector. Concentrations of aldrin as low as 0.001 μg/l were detected. Buescher, et al. (55) employed quantitative and qualitative gas-liquid chromatographic analysis in their studies of the chemical oxidation of pesticides in water. Saturated solutions of lindane, aldrin, and dieldrin were exposed to chlorine, hydrogen peroxide, sodium peroxide, potassium

permanganate, and ozone, and the efficiency of oxidation was evaluated with the gas chromatograph. Buescher and his associates reported that the electron capture detector was satisfactory for the detection and measurement of the pesticides in quantities of 0.001 to 0.005 μg. Warnick and Gaufin (56) outlined in detail a suggested procedure consisting of liquid-liquid extraction followed by gas-liquid chromatographic analysis with an electron capture detector for the detection and measurement of pesticides in water. They advocated the need for the use of a standard procedure for the detection and measurement of pesticides in order to enable the comparison of the results from different investigations.

Chromatography is very useful in the identification of unknown materials when one is looking for a particular compound or series of compounds for which standards are available. However, when the identification of a complex mixture is desired and very limited knowledge as to the origin and nature of the material is available, other types of sophisticated instrumentation must be used in conjunction with gas chromatographic analysis.

Black and Christman (32) utilized infrared analysis in their studies of the chemical characteristics of fulvic acids recovered from colored Florida surface waters. Organic materials were recovered from river and lake water by passing the water through ion-exchange columns and recovering the organics with a brine solution. The color causing materials were extracted from the brine solution with chloroform and divided into humic and fulvic acid fractions by solubility separation. The fulvic acids were then chemically degraded under controlled conditions using a bomb calorimeter and the degradation fractions

analyzed with an infrared spectrophotometer. Seven degradation products were identified, all of which were aromatic compounds with aliphatic side chains.

Midwood and Felbeck (29) reported the characterization of yellow organic matter recovered from lake water using infrared analysis. Four hundred liters of lake water were evaporated under reduced pressure, and the resulting concentrate was freeze-dried to extract the soluble organic matter. The organics were fractionated into humic and fulvic acids which were then subjected to infrared analysis in an attempt to determine the compounds present. These investigators reported that most of the spectra showed adsorption bands at wavelengths of 2.94, 3.35, 6.20, and 7.1 microns, representing alcoholic and phenolic hydroxyl groups, methane and methylene groups, alkene and ionized carboxyl groups, and salts of carboxylic acids, respectively.

Infrared spectroscopy was also used by Tengonciang (37) to study the effects of biodegradation on the structure of trace organics. The biodegradability of the extracts was investigated by means of 20 day BOD studies using 5 mg/l solutions of the organics. The author found that the organics were resistant to biological degradation and reported that only very slight changes in the spectra resulted following the 20 day period. Bands at wavelengths of 13.2 and 13.9 microns present before oxidation were absent after the 20 days of biological activity. Tengonciang suggested that the bands could have been due to a phenolic substance which might have been biochemically oxidized.

Grigoropoulos and Ryckman (13) also employed infrared spectrophotometric studies to evaluate the effects of chlorine and chlorine dioxide on chloroform soluble organics. Their studies were conducted at a pH of 10 and with

ratios of CCE to chlorine and CCE to chlorine dioxide of 1 to 10 and 1 to 4, respectively. The CCE was found resistant to chemical oxidation with only a limited portion totally oxidized. On the basis of the infrared data, Grigoropoulos and Ryckman concluded that partial oxidation and limited chlorination and substitution occurred when the organics were exposed to the oxidants.

Fluorescence spectral analysis was utilized by Borneff and Fischer (38) to identify polycyclic aromatic hydrocarbons in organics recovered from river water by carbon adsorption. Paper and column chromatography were used to separate the trace organics prior to the spectral analysis. This procedure has also been employed by Borneff and Fischer (40) to measure quantitatively the amount of carcinogenic 3,4-benzopyrene destroyed by sunlight, and by Borneff and Kunte (39) to evaluate the effectiveness of conventional waste treatment facilities in removing 3,4-benzopyrene and related fluorescent compounds from domestic and industrial wastes. Borneff and his coworkers have pointed out that fluorescence spectral analysis was very suitable for the identification of specific fluorescent compounds, but would not detect nonfluorescing materials.

The information which can be obtained from the infrared analysis of a complex mixture is limited. Likewise, identification by gas chromatographic techniques requires a knowledge of the nature and origin of the sample and, in general, is a trial and error procedure. Feldstein (57) has discussed the advantages of both infrared analysis and gas-liquid chromatography as individual identification procedures when satisfactory isolation or separation techniques are used prior to analysis. The author suggested that an even better use of gas-liquid chromatography could be made if the chromatograph were employed

to separate a complex mixture and the fractions were collected and analyzed by infrared techniques. The combination of gas chromatographic separation and infrared spectrophotometric identification has also been recently suggested by Ryckman, et al. (58, 59) in a review of the origin and methods of detection of trace organics in water.

A summary of the various characterization and identification methods is presented in Table 6 with the applications for which each is best suited. The capacity of the various methods for serving a particular application is denoted by a +, a -, and a $\pm$ for effective, ineffective, and limited application, respectively. Based on the results reported in the literature, the most promising technique for the identification of organic micropollutants is the combination of gas-liquid chromatography and infrared spectroscopy.

Table 6

Characterization and Identification Procedures for Organic Micropollutants

Procedure	Application*						Application for Which It Is Best Suited
	Analysis		Function		Sample Suited for		
	Qualitative	Quantitative	Fractionating	Monitoring	Gross	Purified	
Solubility Separation	±	-	+	-	+	±	General characterization, fractionation prior to identification.
Elemental Chemical Analysis	+	+	-	-	-	+	Determination of specific chemical elements, identification.
Chemical and Biochemical Oxygen Demand Determinations	-	+	-	+	+	+	General characterization, indication of chemical and biological stability.
Chromatography							
Paper	+	±	+	-	±	+	Identification, fractionation prior to identification.
Column	±	-	+	-	+	+	Fractionation prior to identification.
Thin-Layer	+	±	+	-	+	+	Identification, fractionation prior to identification.
Gas-Liquid	+	+	+	±	+	+	Identification, fractionation prior to identification.
Spectroscopy							
Fluorescence	+	±	-	-	-	+	Identification.
Infrared	+	±	-	±	+	+	Identification, characterization.

*Capability of serving a particular application denoted as effective +, ineffective -, limited ±.

III. RECOVERY AND GENERAL CHARACTERIZATION OF ORGANIC MICROPOLLUTANTS

The trace organic materials employed in this investigation were obtained from three subsurface waters and a treated surface water using the carbon adsorption method. These materials were characterized by several procedures, including solubility partitioning, elemental chemical analysis, and organic carbon and chemical oxygen demand determinations; and the results obtained are reported in this chapter. In addition, acute and long-term toxicity studies and identification studies were performed; and the results are presented in the following chapters.

A. SOURCES OF ORGANIC MICROPOLLUTANTS

Three subsurface water sources, Meramec Spring and two deep wells in Rolla, and a treated surface water source, Missouri River at the Central Plant of the St. Louis County Water Company were sampled to obtain materials for this study. The water of Meramec Spring, one of the largest springs in Missouri, is believed to originate for the most part in the immediate drainage area and to be contaminated by surface water (60); consequently, the spring flow varies widely, fluctuating with the precipitation on the drainage area. One of the deep wells was located on the UMR campus, and it had not been in use at the time of sampling because it had shown evidence of contamination; permission was obtained to pump this well continuously specifically for the purposes of this investigation. The other deep well was Well No. 3, one of the nine wells employed by the City of Rolla for its water supply. The Rolla well was necessarily sampled intermittantly as it was placed in operation; therefore, an extended

sampling period was required. The Missouri River water was treated by softening with lime and presedimentation, chlorination, coagulation with ferric salts and sedimentation, rapid sand filtration, postchlorination, and fluoridation; the treated water was sampled continuously for a period of one year at a point just before postchlorination (61). Data pertaining to all four sources are presented in Table 7.

B. RECOVERY OF ORGANIC MICROPOLLUTANTS

Organic micropollutants were recovered from water using the carbon adsorption method (CAM). Three large capacity activated carbon filters in series were employed to concentrate the trace organics from the three subsurface waters. Each unit contained a 0.75 cubic foot central layer of fine carbon and two end layers, each 0.38 cubic feet, of coarse carbon. Large volumes of water, 130,000 to 260,000 gallons, were passed through the filters at a low filtration rate, 4.9 to 6.8 gpm. No pretreatment of the water before filtration was necessary, and the water was passed through all filter units at its natural pH. After an appropriate volume of water had been filtered, the carbon was removed from the filters, dried, and sequentially eluted with chloroform, ethanol, acetone and benzene, or benzene and acetone. Two filter runs were made at Meramec Spring and one each at the two wells. The recovery equipment and procedure have been discussed in detail in the author's Master's thesis (10) and are briefly described in Appendix A. Surface water organic micropollutants were obtained from the St. Louis County Water Company (61). A standard PHS (16, p. 215) activated carbon filter, containing 0.073 cubic feet of carbon (50 per cent fine and 50 per cent coarse) was used to recover these

Table 7

Source and Sampling Data Pertaining to the Recovery
Of Organic Micropollutants from Missouri Water

Location	Source Data			Sampling Data			
	Rock Formation	Depth ft.	Flow or Capacity	Sampling Period	Days	Rate gpm	Volume gal.
Meramec Spring							
Run #1	Van Buren	--	120 cfs ave.* 400 cfs max.	12/23/65-1/ 9/66	17	5.46	129,000
Run #2	Dolomite	--	89 cfs min.	1/ 9/66-1/28/66	18	4.87	133,000
UMR Well	Potosi Limestone	1,150	336 gpm w/26 ft. drawdown**	1/17/66-2/ 1/66	15	6.84	142,000
Rolla Well	Lamotte Sandstone	1,745	400 gpm w/33 ft. drawdown	3/26/66-5/31/66	115†	6.23	262,000
Missouri River at St. Louis	--	--	78,600 cfs ave.* 676,000 cfs max. 4,200 cfs min.	1/ 1/66-12/31/66	365	0.22	115,492‡

*Data from USGS, Water Resources Division, Rolla, Mo. (60).
**Data from Missouri Geological Survey and Water Resources, Rolla, Mo. (62).
†Pump operated 38 days (equivalent to 35 24-hr. days) during this period.
‡Total volume filtered in 24 biweekly samples (61).

materials from the treated Missouri River water. Relatively small volumes of water, 3,750 to 6,600 gallons, were filtered in regular biweekly periods at a rate proportional to the water production of the treatment plant. At the end of each two week period, the carbon was replaced in the filter and the previously used carbon was dried and extracted with chloroform to recover the organics. Twenty-four consecutive samples collected in 1966 were obtained from the St. Louis County Water Company and combined to give the yearly composite employed in this study. Pertinent sampling data for all sources are given in Table 7.

The concentrations of the organic micropollutants recovered from the various sources are summarized in Table 8. It should be pointed out that the quantity of acetone and benzene soluble organics for all the units, except Meramec Spring Run #1 Unit #1, was obtained by extracting a known volume of the carbon (20 to 30 per cent) and estimating the total quantity present. All the carbon of Unit #1 of Meramec Spring Run #1 was eluted with these solvents. As can be seen from the data given in Table 8, one unit was sufficient to recover all the measurable CCE* and CAE* materials present in the deep well waters, while CAcE* and CBE* materials were recovered from all three units. In contrast to this, significant quantities of CCE and CAE were recovered with each of the three filters used in the two runs at Meramec Spring. The concentration of CAcE and CBE found in the Meramec Spring water, when compared on the basis of the corresponding CCE and CAE concentrations, was considerably

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*CCE: Carbon Chloroform Extract; CAE: Carbon Alcohol Extract; CAcE: Carbon Acetone Extract; CBE: Carbon Benzene Extract.

Table 8

Concentrations of Organic Micropollutants
Recovered from Missouri Waters

Location	Unit No.	Organic Extracts, μg/l			
		Chlorof.	Ethanol	Acet./Benz.	Benz./Acet.
Meramec Spring Run #1	1	30.4	47.6	24.5/0.0	10.0/10.8
	2	10.8	39.6	28.2/0.0	12.9/10.2
	3	2.5	21.8	18.8/0.0	8.8/ 5.5
	Total	43.7	109.0	71.5/0.0	31.7/26.5
Meramec Spring Run #2	1	51.7	76.4	4.0/0.8	2.0/ 1.6
	2	21.8	73.3	9.9/1.0	6.8/17.4
	3	18.6	45.8	15.8/5.6	2.6/14.5
	Total	92.1	195.5	29.7/7.4	11.4/33.5
UMR Well	1	4.8	8.0	3.5/1.4	0.0/18.5
	2	0.0	0.0	2.5/1.0	0.0/15.4
	3	0.0	0.0	2.0/1.0	0.0/12.6
	Total	4.8	8.0	8.0/3.4	0.0/46.5
City of Rolla Well	1	0.1	2.1	0.2/0.1	1.2/ 1.4
	2	0.0	0.0	0.2/0.2	1.1/ 1.2
	3	0.0	0.0	0.2/0.1	1.1/ 1.1
	Total	0.1	2.1	0.6/0.4	3.4/ 3.7
Missouri River at St. Louis	-	36.2*	--	--	--

*Average of 24 biweekly samples; individual values ranged from 17 to 71 μg/l.

smaller than the concentration of the CAcE and CBE found in the well waters. This points out that acetone and benzene were better elutants for the recovery of organics from deep well waters by the carbon adsorption technique than were chloroform and ethanol which have been widely used with surface waters. It is interesting to note that while the concentrations of the CCE and CAE found in the spring water were only slightly less than those reported by Sproul and Ryckman (6) for a Missouri surface water (98 μg/l CCE and 230 μg/l CAE), the CCE and CAE concentrations were in all cases significantly less than those reported by Robinson, et al. (8) for Illinois ground waters (706 μg/l total CCE and 1,750 μg/l total CAE). The concentration of the CCE in treated Missouri River water was lower than the values reported by Sletten (46) for St. Louis tap water (average 57.2 μg/l CCE, range 36.8 to 75.4 μg/l).

It should be pointed out that the quantities of organic micropollutants recovered in this study are conservative as a consequence of the limitations of the CAM; that is, not all the organics in the water are adsorbed on the carbon, and not all the adsorbed materials are eluted from the carbon with the solvents. Although other methods have been advocated for the recovery of trace organics from water, the carbon adsorption method is the only one which allows sampling of large volumes of water and recovering of enough material for characterization and toxicity studies.

C. STOCK SOLUTIONS

The majority of the studies performed with the organic micropollutants required that these materials be in solution. Aqueous stock solutions of the

CCE and CAE materials were made using a VirTis mixer*; a measured amount of organic extract was placed in a deep fluted mixing flask with a known volume of water and then mixed at about 8,000 rpm until all the material was in solution. Due to the limited solubility of the extracts and the need for solutions of higher concentration, a Rinco vacuum evaporator** was employed to concentrate the stock solutions. Evaporation was accomplished by applying vacuum with a water aspirator and heating the solution at 45 to 50°C in a water bath.

D. GENERAL CHARACTERIZATION OF ORGANIC MICROPOLLUTANTS

The organic micropollutants were characterized by elemental chemical analysis, chemical oxygen demand and carbon determinations, and solubility partitioning. Although some of the results of these studies have been reported previously (7,10), general characterization data are summarized in this section in order to provide a measure of the character of the complex organics using parameters commonly employed in the sanitary engineering field.

1. Elemental Chemical Analysis

Elemental chemical analyses were performed on several samples by a commercial laboratory*** and are presented in Table 9 with the corresponding empirical formulas.

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*Aero-Seal Chemixer, Model No. 26-400, a product of the VirTis Company, Inc., Gardiner, N. Y.

**Model No. VE-1000B, a product of the Rinco Instrument Company, Inc., Greenville, Ill.

***Micro-Tech Laboratories, Inc., 4117 Oakton Street, Skokie, Ill.

Table 9

Elemental Composition and Empirical Formulas
Of Organic Micropollutants Recovered from Missouri Waters

Extract	Elemental Composition, %						Empirical Formula
	C	H	O	S	P	N	
Meramec Spring Run #1 Unit #1							
CCE	59.0*	6.96*	24.2*	< 0.1	0.36	0.68	$C_{4.5}H_{6.8}O_{1.5}N_{0.05}P_{0.01}$
CAE	51.2*	6.52*	36.0*	< 0.1	0.35	2.53	$C_{4.2}H_{6.4}O_{2.4}N_{0.18}P_{0.01}$
Meramec Spring Run #2 Unit #2							
CCE	62.5	7.40	24.0	0.20	0.22	0.54	$C_{5.2}H_{7.4}O_{1.5}N_{0.04}P_{0.01}S_{0.01}$
CAE	46.3	7.47	36.6	0.13	2.80	2.04	$C_{3.9}H_{7.5}O_{2.3}N_{0.14}P_{0.09}$
UMR Well Unit #1							
CCE	52.5*	6.74*	14.7	27.8*	0.22	0.32	$C_{4.2}H_{6.4}O_{0.92}N_{0.02}P_{0.01}S_{1.0}$
CAE	42.9	6.81	36.0	< 0.1	0.24	1.36	$C_{3.6}H_{6.8}O_{2.3}N_{0.10}P_{0.01}$
Missouri River at St. Louis							
CCE	67.2	7.78	18.5	1.36	0.10	1.68	$C_{5.6}H_{7.8}O_{1.2}N_{0.12}S_{0.04}$

*Average of analysis on two separate samples.

2. Organic Carbon and Chemical Oxygen Demand

The organic carbon content of the various extracts was determined with a Beckman Carbonaceous Analyzer* (Figure 1). To determine the organic carbon, a 15 ml sample of a stock solution of trace organics was first acidified to a pH of 2 with concentrated hydrochloric acid and purged with nitrogen** for five minutes to remove any inorganic carbon which might have been present. A 20 μl volume of the purged sample was accurately delivered into the combustion tube of the analyzer using a Hamilton Microliter syringe.*** The sample was vaporized immediately, and the organic components were oxidized to carbon dioxide and water in a stream of pure oxygen† and at a temperature of 960^{o}C. The gas stream was passed through an infrared analyzer sensitized to carbon dioxide and the output of the analyzer was recorded on a strip chart. The organic carbon concentration was then determined from a calibration curve relating pen response to carbon concentration and was expressed as a per cent of the organic material. The instrument was calibrated with a solution of glacial acetic acid of known strength.

The results of the carbon determinations are presented in Table 10 and represent the average of several analyses; also reported are the values obtained for selected extracts from elemental chemical analyses. As can be

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*Model No. 137879Y, a product of Beckman Instruments, Inc., Fullerton, Calif.

**High purity grade nitrogen procured through the Central Stores, University of Missouri - Columbia, Columbia, Mo.

***Model 705 N/LT W/G, a product of the Hamilton Company, Whittier, Calif.

†Two grades of oxygen were used: Ultra High Purity, purchased from the Matheson Company, Jolliet, Ill., and water pumped, procured through the Central Stores, University of Missouri - Columbia, Columbia, Mo.

Figure 1

Beckman Carbonaceous Analyzer

Table 10

Carbon Content and Oxygen Demand of Organic Micropollutants Recovered from Missouri Waters

Extract	Carbon Content %		TOD	COD	
	E.C.A.*	B.C.A.**	mg O_2/mg extract	mg O_2/mg extract	% of TOD
Meramec Spring Run #1 Unit #1					
CCE	59.0	58.0	1.85	1.37	74.0
CAE	51.2	49.6	1.52	1.49	98.0
Unit #2					
CCE	--	55.0	--	1.48	--
CAE	--	51.6	--	1.30	--
Unit #3					
CCE	--	57.0	--	1.35	--
CAE	--	53.0	--	1.40	--
Meramec Spring Run #2 Unit #1					
CCE	--	60.5	--	1.32	--
CAE	--	50.5	--	1.31	--
Unit #2					
CCE	62.5	62.0	2.01	2.00	99.5
CAE	46.3	46.0	1.48	1.40	94.5
Unit #3					
CCE	--	56.2	--	1.72	--
CAE	--	61.2	--	1.94	--
UMR Well					
CCE	52.5	53.0	1.72	1.64	95.3
CAE	42.9	44.0	1.55	1.46	94.2
Missouri River at St. Louis					
CCE	67.2	68.0	2.39	2.00	84.0

*Based on the elemental analysis.

**Based on analysis with the Beckman Carbonaceous Analyzer.

seen from the data in this table, the carbon contents determined by the two methods compared very favorably.

The chemical oxygen demand was determined for each extract using the dilute dichromate method outlined in Standard Methods (16, p. 512). A mixture consisting of 5 ml stock solution, 5 ml 0.025 N potassium dichromate, 10 ml concentrated sulfuric acid containing 9.3 g/l silver sulfate, and a few pumice stones was refluxed for two hours. It was then allowed to cool, diluted to 100 ml with distilled water, and the remaining potassium dichromate was titrated with 0.01 N ferrous ammonium sulfate using ferroin indicator. A reagent blank containing distilled water instead of sample was also run. The COD values, expressed as mg oxygen per mg extract, are presented in Table 10 and are the average of two determinations. Also given in this table are theoretical oxygen demand values computed for selected extracts from the corresponding elemental chemical analyses on the basis of complete oxidation to carbon dioxide and water. The COD values for the various extracts ranged from 1.30 to 2.00 mg oxygen per mg extract and, in most cases, represented more than 90 per cent of the TOD values.

3. Group Breakdown

Solubility partitioning was used to separate the organic micropollutants into various groups based on their solubility in ether under different conditions of pH. The method described by Shiner, et al. (35, p. 101) was employed for these separations and is shown diagrammatically in Figure 2. The various fractions obtained by solubility partitioning were the ether insolubles, water solubles, weak acids, strong acids, bases, amphoterics, and neutrals. The neutral

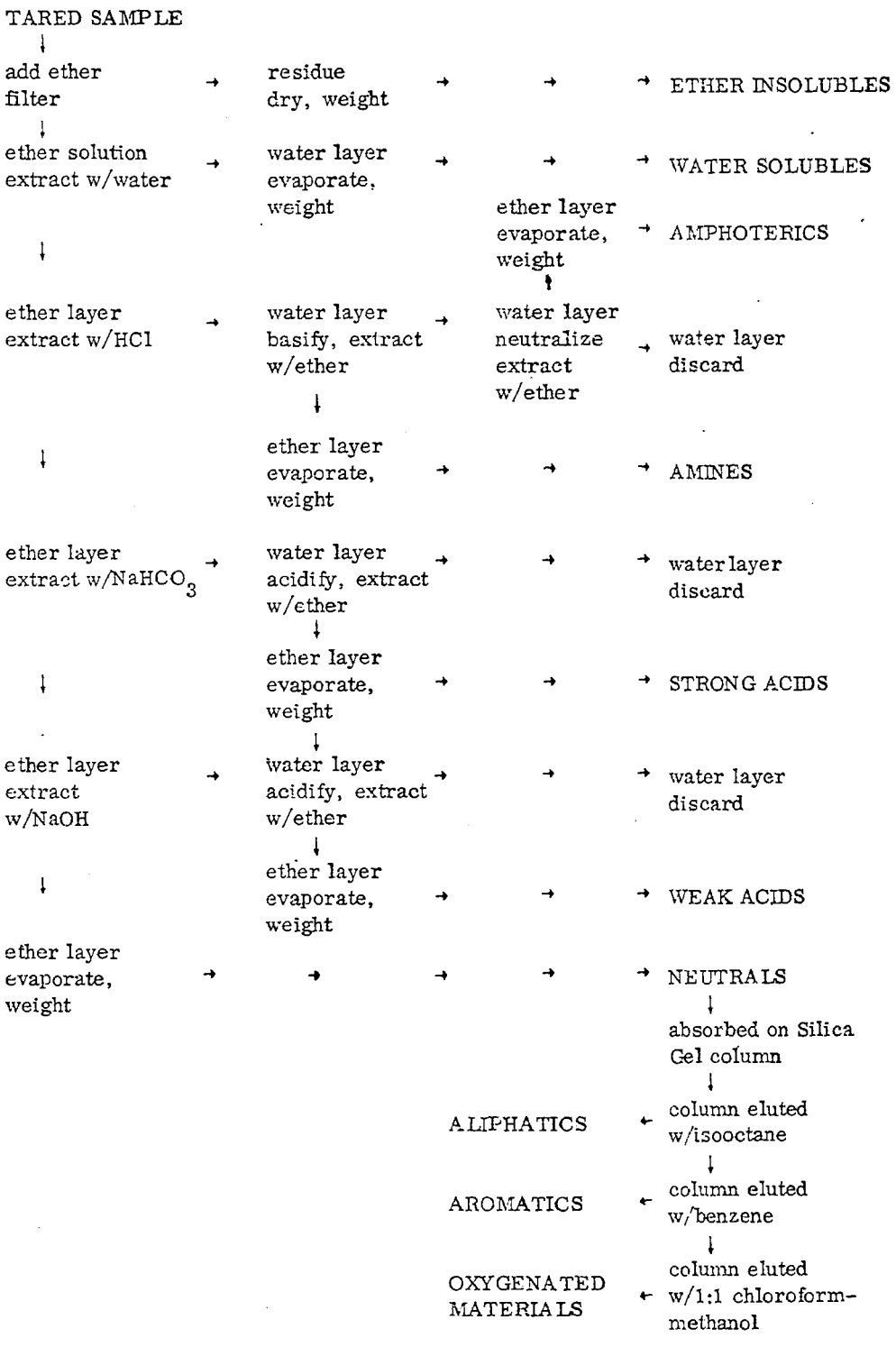

Figure 2

Solubility Partitioning and Column Chromatographic Separation Of Organic Micropollutants Recovered from Missouri Waters

fraction was further separated by column chromatography (Figure 2) into aliphatics, aromatics, and oxygenated materials.

Average values from two separations on each extract are presented in Table 11. The CAE from the first unit of both spring runs and the UMR well were not ether soluble and no further separation of these materials could be obtained; however, the CAE from Units #2 and 3 of both spring runs were partially ether soluble. Four separations were performed on each alcohol soluble material to validate this difference. The major group in the CAE which was separated was the ether insolubles. The predominating group in the CCE from the spring and the river water was the water solubles, except for the extracts recovered with the third unit at the spring; for these extracts, as well as for the CCE, CAcE, and CBE from the UMR well, the neutrals were the major group. The oxygenated fraction predominated in the neutrals of all the CAE materials, the CCE recovered with Units #1 and 2 of spring Run #1, and the CCE in the treated river water. On the other hand, the aliphatic fraction predominated in the neutrals of the CCE materials in the UMR well water and the CCE recovered with the third unit of both runs at the spring. It should be pointed out that Myrick and Ryckman (9) reported the group breakdown of raw Missouri River water CCE, but were unable to separate the corresponding CAE.

Group breakdown of the trace organics separated the complex materials into groups of a less complex nature. These fractions were found very useful in the identification phase of this investigation which is reported in Chapter V.

Group Breakdown of Organic Micropollutants Recovered from Missouri Waters

Test Material	Fraction, %											
	Ether Insolubles	Water Solubles	Weak Acids	Strong Acids	Bases	Amphoterics	Neutrals					Others
							Total	Aliphatics*	Aromatics*	Oxygenated*	Others*	
Meramec Spring Run #1 Unit #1												
CCE	10.0	31.0	8.5	11.8	0.77	0.91	11.8	16.1	36.9	37.0	10.0	25.2
CAE	>99.0	--	--	--	--	--	--	--	--	--	--	--
Unit #2												
CCE	5.0	42.0	7.4	12.8	0.40	0.60	14.2	4.2	45.5	41.7	8.6	17.6
CAE	77.0	10.6	0.8	4.4	0.00	0.00	1.5	11.1	1.0	69.3	18.6	5.0
Unit #3												
CCE	6.9	13.5	14.0	7.0	0.00	0.00	48.0	50.8	5.0	25.4	18.8	10.6
CAE	44.0	33.0	2.5	9.5	0.20	0.20	3.1	16.2	0.0	56.7	27.1	7.5
Meramec Spring Run #2 Unit #1												
CCE	2.1	27.0	13.6	13.4	0.83	0.35	14.0	22.0	24.5	50.5	3.0	28.7
CAE	>99.0	--	--	--	--	--	--	--	--	--	--	--
Unit #2												
CCE	1.2	30.0	9.6	10.6	1.00	0.90	23.0	6.8	21.0	44.3	27.9	23.7
CAE	62.4	15.7	2.0	11.7	0.00	0.00	3.6	10.0	0.7	83.0	6.3	4.6
Unit #3												
CCE	6.7	16.8	11.5	25.1	1.30	0.00	28.3	49.6	4.2	27.7	18.5	10.0
CAE	58.0	14.7	1.2	8.0	0.00	0.00	1.6	9.1	4.3	79.9	6.7	16.5
UMR Well												
Unit #1												
CCE	6.4	15.0	6.8	11.5	1.50	1.60	21.4	48.5	19.6	21.6	10.3	35.8
CAE	>99.0	--	--	--	--	--	--	--	--	--	--	--
CAcE-1**	1.6	5.0	8.3	5.0	0.60	0.50	71.0	0.0			1.0	8.0
CBE-2**	0.0	0.6	2.2	0.6	0.50	0.20	83.5	5.0	56.2	25.4	13.4	12.4
Missouri River at St. Louis												
CCE	2.9	25.6	10.2	5.5	2.10	1.00	21.4	11.2	14.7	74.1	0.0	30.0

*% of total neutral fraction.

**The numbers 1 and 2 refer to the order in which acetone and benzene were employed.

IV. TOXICITY STUDIES

The acute and long-term toxic effects of the organic micropollutants recovered from both surface and subsurface waters were studied using batch-type bioassays with fish as the test animal. The toxic effects of two pesticides, sevin and malathion, were also evaluated. In addition to the gross lethal effect of the test materials, their effect on fish organs was also of concern especially in evaluating the mode of action of the toxicants.

Toxicity studies were performed using rainbow trout (Salmo gairdnerii), blue green sunfish (Lepomis cyanellus), red shiners (Notropis lutrensis), golden shiners (Notemigonus crysoleucas), and mosquitofish (Gambusia affinis affinis). All the test fish were employed in the acute studies, but only trout and red shiners were used in the long-term studies. The trout, sunfish, and mosquitofish were obtained from the Missouri Conservation Commission; and the shiners were secured from local hatcheries. The fish were transported to the laboratory in five gallon polyethylene bottles with ice and oxygen added to keep the water temperature low and to maintain an adequate dissolved oxygen level. The trout were maintained in a constant temperature (12°C) walk-in room, while the sunfish, shiners, and mosquitofish were kept in the laboratory (21°C). The fish were acclimated in 15 gallon glass aquaria for a period of at least 10 days. These aquaria were equipped with an aeration apparatus consisting of either a 2 foot length of 0.25 inch diameter polyethylene tubing with 6 pin holes or a diffusion stone through which air was pumped with a Precision air-vacuum pump.* The fish were fed Purina trout chow each day except during the test

*Model PV-35, a product of the Precision Scientific Co., Chicago, Ill.

period and the 48 hours preceding it. The aquaria were cleaned with soap and water every day. Terramycin* (4 mg/l) was added to the holding aquaria each day, and periodic formalin baths (1 ml 25 per cent formalin per gallon of water for one hour) were used to suppress biological growths on the fish.

The water employed in the bioassay studies was UMR tap water which had been aerated in 5 gallon Pyrex glass bottles for at least 48 hours in order to remove any chlorine and precipitate any iron that might have been present. General characteristics of the aerated tap water are summarized in Table 12.

A. ACUTE STUDIES

Since the approximate toxicity of the trace organics and pesticides to the test fish was not known, exploratory bioassay studies were conducted (16, p. 551) using test solutions which contained individual CCE or CAE, CCE and CAE combined in their naturally occurring proportions, and the two pesticides, sevin** and malathion.*** Three liter volumes of water containing the test materials in one gallon aerated test containers were used in the exploratory studies. A control system was also used and contained only dilution water. Three acclimated fish were placed in each container and observed periodically over a 24 hour period.

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*5.2 per cent active ingredient, a product of the M-F-A Company, Columbia, Mo.

**50 per cent active ingredient, a product of the Union Carbide Corporation, New York, N. Y.

***57 per cent active ingredient, a product of the M-F-A Company, Columbia, Mo.

Table 12

Characteristics of Aerated UMR Tap Water
Used in Bioassay Studies

Characteristic	Concentration			Determination or Instrument Employed
	Ave.	Max.	Min.	
pH, units	7.6	8.2	7.4	Beckman Zeromatic pH meter with glass electrode
Alkalinity, mg/l as $CaCO_3$	130	160	100	Acid Titration (16, p. 48)
Hardness, mg/l as $CaCO_3$				
Total	140	170	95	EDTA Titration (16, p. 147)
Calcium	70	89	46	
Dissolved Oxygen, mg/l	6.9	7.1	6.7	Galvanic Cell Oxygen Analyzer
Ammonia Nitrogen, mg/l	5.0	6.2	4.0	Direct Nesslerization (16, p. 193)
Temperature, ^{o}C				
Trout Studies	12.2	13.0	12.0	Temperature probe on Galvanic Cell Oxygen Analyzer
Sunfish, Minnow, Mosquitofish Studies	21.0	21.5	20.0	
Total Chlorine, mg/l	<0.1	<0.1	<0.1	Ortotolidine Arsenite (16, p. 441)

On the basis of the results of the exploratory tests, an appropriate range of concentrations was selected for the bioassay studies. These concentrations constituted a logarithmic series as recommended in Standard Methods (16, p. 553). Five test samples were prepared, and a control unit was used to detect any disease or physical unfitness of the test fish or faulty procedure. Five gallon test containers containing 18 liters of test solution were used in the trout and sunfish studies, and one gallon test containers containing 3 liters of test solution were employed in the shiner and mosquitofish studies; all units were aerated using the Precision air-vacuum pump. Six trout and eight sunfish, shiners, or mosquitofish were transferred to each test container. The fish were observed continuously for the first 2 to 4 hours of the test depending on their reaction. Thereafter, the observations were periodic but often enough to determine the number of fish killed at the end of 24, 48, 96, and 120 hours.

The results of the acute bioassay studies are shown in Table 13. Individual subsurface water CCE and CAE materials were not toxic even at concentrations approaching the upper limit of their solubility (270 and 400 mg/l for a CCE and CAE, respectively). However, in many cases when the CCE and CAE were combined in the same proportion as they were recovered from the water, the combined extract was toxic to the test fish. The extent of the toxicity of the subsurface water organics depended on both the species and size of the fish and the organic micropollutant being tested. The small trout (5.0 cm) were much more resistant to the trace organics than were the larger trout (10.5 cm); the 24 hour TLm values for 5.0 and 10.5 cm trout exposed to Meramec Spring Run #2 Unit #2 combined extracts were 201 and 88 mg/l, respectively.

Table 13

Acute Toxicity of Organic Micropollutants Recovered from Missouri Waters

Test Material	Ratio CCE/CAE	Test Fish (Length, cm./Weight, g)	TLm Value, mg/l 24 hr.	48 hr.	96 hr.	120 hr.
Meramec Spring Run #1						
Unit #1	1/1.56	Trout (9.4/9.7)	138	130	96	92
		Red Shiners (5.0/2.0)	no effect up to 240			
Unit #2	1/3.65	Red Shiners (2.9/1.3)	no effect up to 200			
Meramec Spring Run #2						
Unit #1	1/1.48	Trout (10.3/16.3)	130	125	95	82
		Red Shiners (2.8/1.2)	no effect up to 305			
		Red Shiners (5.6/2.2)	no effect up to 305			
		Sunfish (7.5/9.1)	166	141	115	103
Unit #2	1/3.35	Trout (10.5/16.0)	88	75	61	56
		Trout (5.0/2.6)	201	186	155	130
		Red Shiners (5.7/2.3)	195	170	148	120
		Golden Shiners (6.4/4.0)	180	171	160	152
		Sunfish (7.0/8.7)	137	121	114	100
		Mosquitofish (2.0/0.2)	no effect up to 270			
Unit #3	1/2.46	Trout (10.1/16.1)	no effect up to 180			
		Trout (5.2/2.0)	no effect up to 180			
Missouri River at St. Louis						
CCE	N/A	Trout (5.3/2.8)	36	32	28	24
		Golden Shiners (6.6/4.3)	59	52	39	33
		Sunfish (7.7/11.7)	56	49	45	39
Sevin**	N/A	Trout (10.0/15.0)	2.3	1.5	1.0	0.7
		Red Shiners (4.6/1.4)	13	12	9.2	7.4
Malathion***	N/A	Trout (10.0/15.0)	0.0050	0.0046	0.0028	0.0023
		Red Shiners (4.8/1.9)	0.0400	0.0360	0.0250	0.0230

*Combined extract; **based on 50% active ingredient; ***based on 57% active ingredient.

The relative resistance of the test fish exposed to Meramec Spring Run #2 Unit #2 combined extracts, calculated on the basis of the 24 hour TLm values and a resistance factor of 1.0 for the larger trout (10.5 cm), was: sunfish (7.0 cm) - 1.56, golden shiners (6.4 cm) - 2.04, red shiners (5.7 cm) - 2.22, small trout (5.0 cm) - 2.28, and mosquitofish (2.0 cm) - 3.10.

The surface water CCE was also toxic to the test fish, but at a significantly lower concentration. The 24 hour TLm of 36 mg/l for trout (5.3 cm) exposed to this extract compares very favorably with the 38 mg/l 24 hour TLm value reported by Sletten (46) for fingerling trout exposed to a CCE recovered from St. Louis tap water. On the basis of a resistance factor of 1.0 for the small trout (5.3 cm) and considering 24 hour TLm values, the relative resistance to the surface water CCE of sunfish (7.7 cm) and golden shiners (6.6 cm) was 1.56 and 1.64, respectively. It should be noted that the small trout were more resistant than either the sunfish or the shiners when exposed to the subsurface water combined extracts.

Both pesticides were toxic to trout and red shiners with malathion being considerably more toxic (Table 13). The pesticide concentrations were based on the active ingredient in the commercial preparation. Evans (63) has reported a 24 hour TLm value of 54 mg/l for western golden shiners (6.5 cm, 2.3 g) exposed to sevin (based on a 50 per cent active commercial preparation). This is equivalent to 27 mg/l based on active ingredient which is over twice as much as the 24 hour TLm value determined for red shiners in this study; this may have been caused by the difference in the species and size of fish.

The fish exposed to lethal levels of the organic micropollutants exhibited a loss of balance, rapid operculum movement, and violent swimming before death. All the test fish which died from exposure to a toxicant were dissected. and the organs examined with the aid of a Bausch & Lomb binocular stereoscopic microscope* and a Bausch & Lomb dynazoom binocular laboratory microscope** equipped with a Polaroid camera. The gills, heart, liver, gas bladder, and intestines were routinely examined. The only organs which appeared to be affected in the fish exposed to subsurface water extracts were the gills which showed a lack of red color and an accumulation of light colored material on the gill lamella, and the gas bladder which was empty completely devoid of air. Because visual observation indicated that the affected fish suffered from lack of oxygen, gill surface area measurements were made on as many test fish as possible, both exposed and unexposed; and the values obtained are shown in Table 14 together with other physical characteristics. The gill surface area was determined as shown in Figure 3 by calculating the average surface area of a lamella and multiplying by the average total number of lamella per fish. Although the fish exposed to toxic levels of surface water CCE exhibited characteristics before death similar to those of the fish exposed to subsurface water extracts, visual examination of the affected organs indicated that the mode of action could have been different. The gills and gas bladder of the fish exposed to the Missouri River extract appeared normal, but extreme hemorrhaging was noted around the heart and liver. Dissection and visual examination of the

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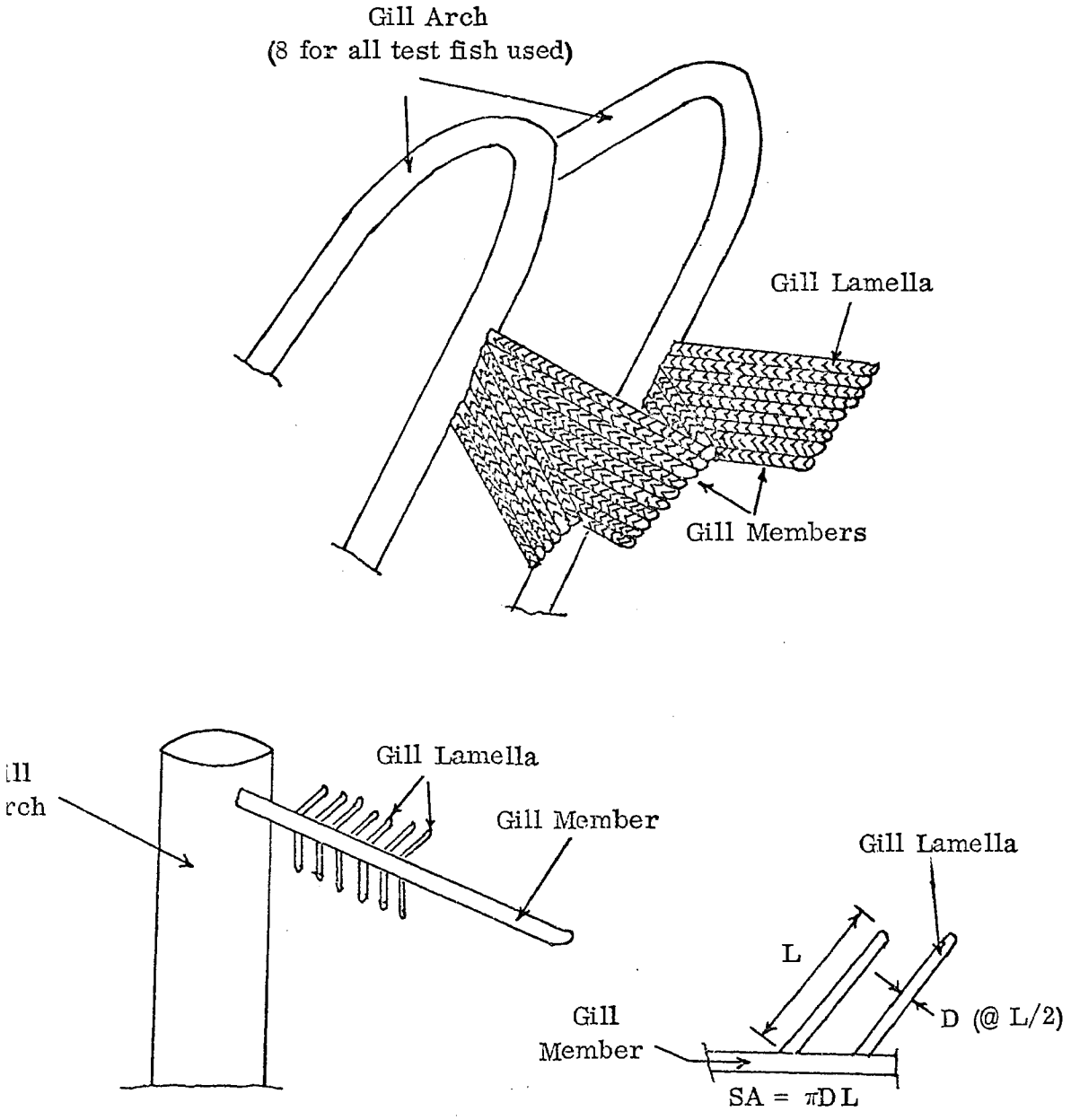
*Model BVB-73, a product of Bausch & Lomb, Incorporated, Rochester, N.Y.
**Model PB-252, a product of Bausch & Lomb, Incorporated, Rochester, N.Y.

Table 14

Physical Characteristics of Test Fish

Test Fish	Number of Fish Observed	Average Fish		
		Length cm	Weight g	Gill Surface Area mm^2
Trout	17	4.9	2.4	2,300
	28	5.0	2.6	2,400
	30	5.2	2.0	2,390
	36	5.3	2.8	2,480
	38	5.3	2.2	2,470
	20	6.0	3.8	2,780
	15	6.3	4.1	2,900
	18	8.2	11.0	4,300
	10	10.3	16.3	3,200
	10	10.5	16.0	3,100
Red Shiners	31	2.9	1.3	2,200
	41	4.6	1.6	2,100
	30	4.7	1.5	2,780
	20	4.8	1.9	2,800
	30	5.6	2.2	2,420
	57	5.7	2.3	2,320
Golden Shiners	20	6.4	4.0	2,480
	18	6.6	4.3	2,500
Sunfish	18	7.0	8.7	1,220
	13	7.5	9.1	1,240
	20	7.7	11.7	1,200



Total Surface Area =

(SA/Lamella) x (No. Lamella/Member) x (No. Members/Arch) x (8 Arches)

Figure 3

Determination of Gill Surface Area

fish killed by the two pesticides revealed that the gills, heart, and liver appeared to be normal.

B. MODE OF ACTION STUDIES

The acute toxicity studies indicated that the fish were dying from a shortage of oxygen and that the surface and subsurface water extracts were affecting different organs. This apparent suffocation of the fish could have been caused by either a physical blockage of the oxygen transfer at the gills or an internal disruption of respiratory enzyme activity. In order to evaluate further the mode of action of the toxicants, enzyme studies using a Warburg respirometer* at 20°C and oxygen transfer studies utilizing a semipermeable membrane were performed.

1. Respiratory Enzyme Studies

Oxygen uptake studies using trout tissue homogenate were employed to evaluate the effect of the organic micropollutants and pesticides on the activity of respiratory enzyme systems. The procedure used was generally similar to that employed by Sletten (46) to evaluate the toxic effect of CCE materials recovered from Missouri River water and by Hiltibrand and Johnson (64) to study the effects of pesticides on the oxygen uptake by mitochondrial enzyme systems. The liver, heart, and gill members were removed from freshly sacrificed trout (12 to 16 inches long) at 5°C, homogenized in a Waring blender,** and suspended and stored in an isotonic solution at 5°C. The suspending medium

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*Model RWBP3, a product of Gilson Medical Electronics, Middleton, Wis.
**Model 1042, a product of the Waring Products Co., Winsted, Conn.

was a modification of Robinson's EDTA isotonic solution as given in Manometric Techniques by Umbreit, et al. (65, p. 133). To prepare this medium, 1,904 ml of Solution 1 and 26.2 ml of Solution 2 were combined, and 165 g sucrose was added to give a 0.25 M solution; EDTA (0.001 M) solution, rather than distilled water, was used in making the individual solutions. The medium was sterilized before use by autoclaving at 121°C, 16 psi, for 15 minutes.

Solution 1		Solution 2	
NaCl (0.154 M)	1776 ml	Na_2PO_4 (0.067 M)	7.7 ml
KCl (0.154 M)	64 ml	KH_2PO_4 (0.067 M)	18.5 ml
$MgSO_4$ (0.154 M)	16 ml	Total Volume	26.2 ml
$CaCl_2$ (0.110 M)	48 ml		
Total Volume	1904 ml		

Concentrations of the trace organics higher than those that could be obtained with the VirTis mixer and the Rinco evaporator (see p. 40) were necessary for the tissue studies. Because of the limited solubility of the organics in water, several solvents were evaluated as dispersing agents for the organics. In addition to its ability to dissolve large quantities of organics, a desirable dispersant needed to be nonbiodegradable, nontoxic, and possess a low vapor pressure. Several different compounds and combinations of compounds were investigated including the cellosolve-surfactant* mixture (1 part cellosolve - 1.22 parts surfactant) employed by Sletten (46). This mixture was found to

*M-14019, a product of the Tretolite Co., St. Louis, Mo.

dissolve large amounts of organics and possess a low vapor pressure; however, it significantly inhibited enzyme activity (maximum inhibition 50 per cent in the case of liver homogenate). Cellosolve and ethylene glycol individually and in combination (1 to 1.22) were evaluated but were found unacceptable because of enzyme activity inhibition. Acetone was also tried but could not be used because of its high vapor pressure. On the advice of the manufacturer of the surfactant (66), this material was individually evaluated as a solvent and found to dissolve the organics, possess a low vapor pressure, and exert little inhibition of enzyme activity (maximum inhibition 10 per cent for liver homogenate). Stock solutions of the trace organics were prepared by first liquifying the surfactant in a shaker water bath* at 40°C, and then using enough liquid surfactant to dissolve a known amount of extract and diluting to the desired concentration with warm water.

In order to obtain significant oxygen uptakes by the enzyme systems, the addition of a substrate as an energy source was considered necessary. Several simple organic compounds, including sucrose, glucose, citric acid, and succinic acid, were evaluated at a 0.1 M test concentration using liver homogenate. Oxygen uptake values resulting from the presence of these substrates are plotted in Figure 4 and are tabulated in Table B-1, Appendix B. Succinic acid caused a rapid rate of oxygen utilization which was essentially complete within 120 minutes. The other substrates produced a slower oxygen utilization which required

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*Magni Whirl Constant Temperature Bath, a product of Blue M Electric Company, Blue Island, Ill.

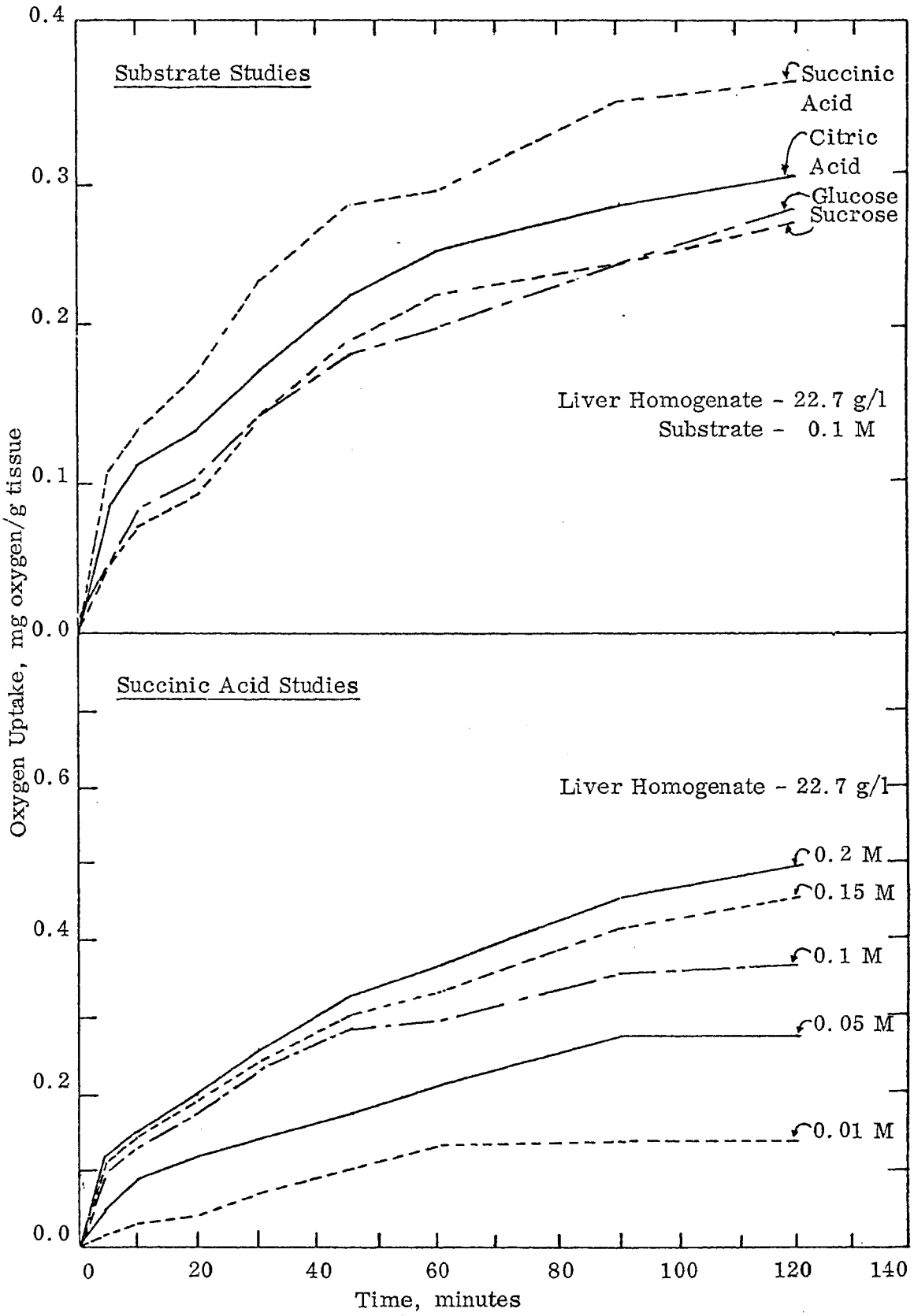

Figure 4

Oxygen Uptake by Trout Liver Homogenate
Exposed to Succinic Acid and Other Substrates

considerably longer than 120 minutes to be completed. Therefore, succinic acid was chosen as the substrate, and a series of experiments were performed to determine a satisfactory concentration. The results of these experiments are presented in Figure 4 and Table B-1, Appendix B. On the basis of these findings, a 0.1 M test concentration of succinic acid was selected.

Enzyme respiration studies were performed at 20°C using the Warburg respirometer and 15 ml reaction vessels with side arms. The flasks were calibrated with mercury as outlined in Manometric Techniques (65, p. 46). To perform a test, one ml each of tissue homogenate and test material were added to the flask, one ml of substrate (0.3 M succinic acid) was placed in the side arm, and 0.1 ml potassium hydroxide (20 per cent) was added to the center well of the flask to absorb carbon dioxide released. The reaction vessels were allowed to equilibrate for 5 minutes, the substrate in the side arm was added to the flask contents, and oxygen uptake readings were taken for a 2 hour period. The effects of several concentrations of subsurface water CCE and CAE materials, individually and in combination, surface water CCE, sevin, and malathion on trout liver (22.7 g/l), heart (11.0 g/l), and gill (6.7 g/l) homogenates were evaluated. Inhibition of enzyme activity was determined by comparing the oxygen uptake of the test mixture with that of a control flask containing surfactant instead of a surfactant-organics mixture.

The oxygen uptake values are presented in Tables B-2, B-3, and B-4, Appendix B, for subsurface water organics, surface water organics, and pesticides, respectively. Organic micropollutants recovered in both runs at Meramec Spring exerted no significant inhibition to any homogenate at the concentrations

studied which ranged from 0.01 to 3.6 mg organics per mg tissue. The effect of the combined spring water CCE and CAE (Run #1 Unit #1) is illustrated in Figure 5; because of the similarity of data, graphs for the organics recovered by the other units and in the other run are not presented. Sevin and malathion, at concentrations of 0.01 to 7.2 mg active pesticide per mg tissue, also did not affect the activity of the enzyme systems. Missouri River water CCE definitely inhibited the enzyme activity of all three homogenates; the effect of this material on oxygen uptake is shown in Figure 6. The median respiratory tolerance limit (RTLm), defined by Sletten (46) as that concentration of toxicant which would reduce the activity of the test solution to 50 per cent of the control, was calculated for each type of tissue preparation as shown in Figure 7 on the basis of the 60 minute oxygen uptake values (Table B-5, Appendix B). The 60 minute RTLm values of the three tissue homogenates exposed to the surface water CCE were as follows:

Tissue Homogenate	Median Respiratory Tolerance Limit mg organics per mg tissue
Liver	0.11
Heart	0.22
Gill	0.38

Sletten (46) has reported for trout liver homogenate in the presence of CCE recovered from St. Louis tap water a 60 minute RTLm value of 0.108 mg organics per mg tissue. This value is essentially the same as the 60 minute RTLm for the trout liver homogenate exposed to the surface water CCE.

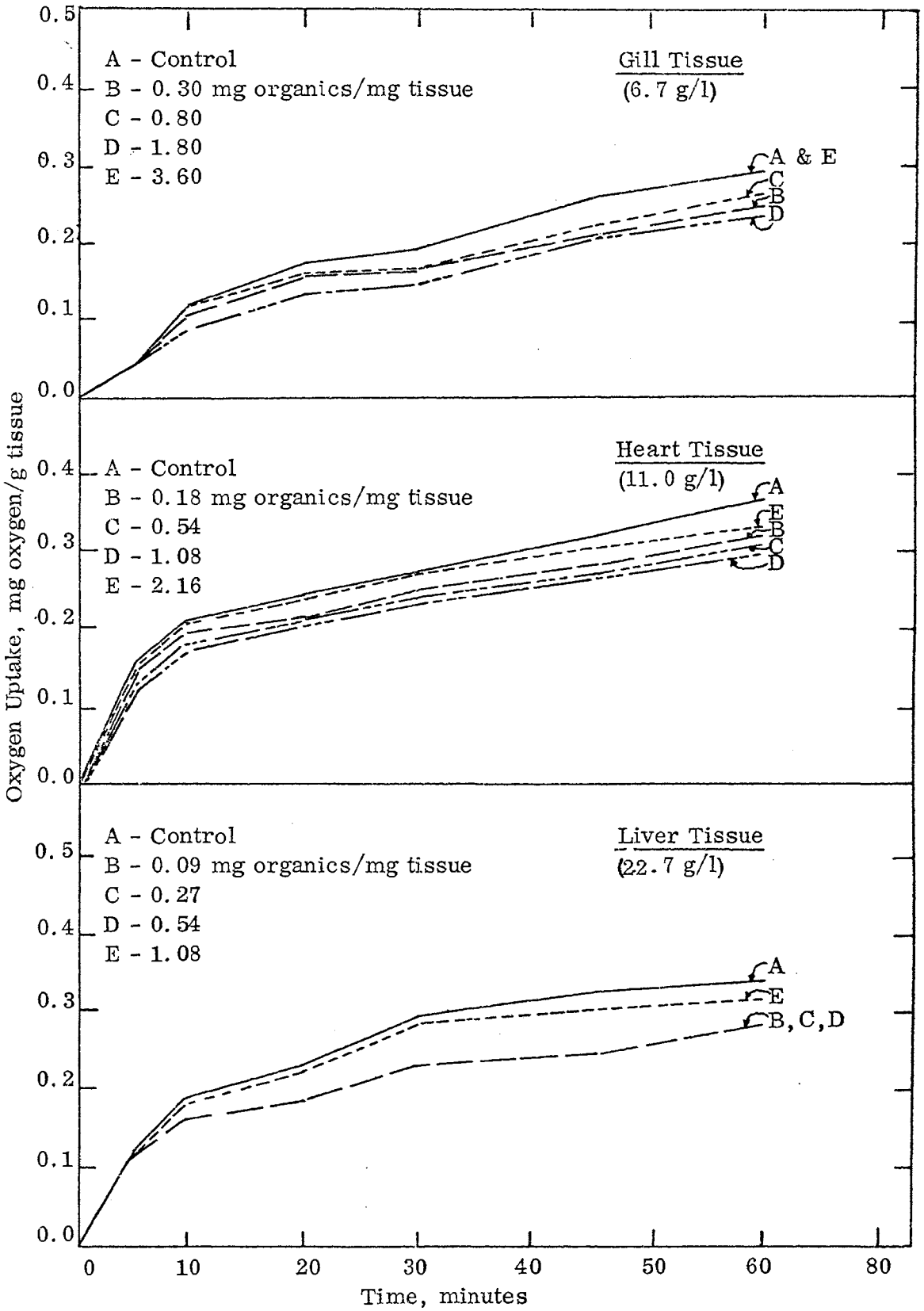

Figure 5

Effect of Meramec Spring Water CCE and CAE (Run #1 Unit #1) On Trout Homogenate Respiratory Enzyme Activity

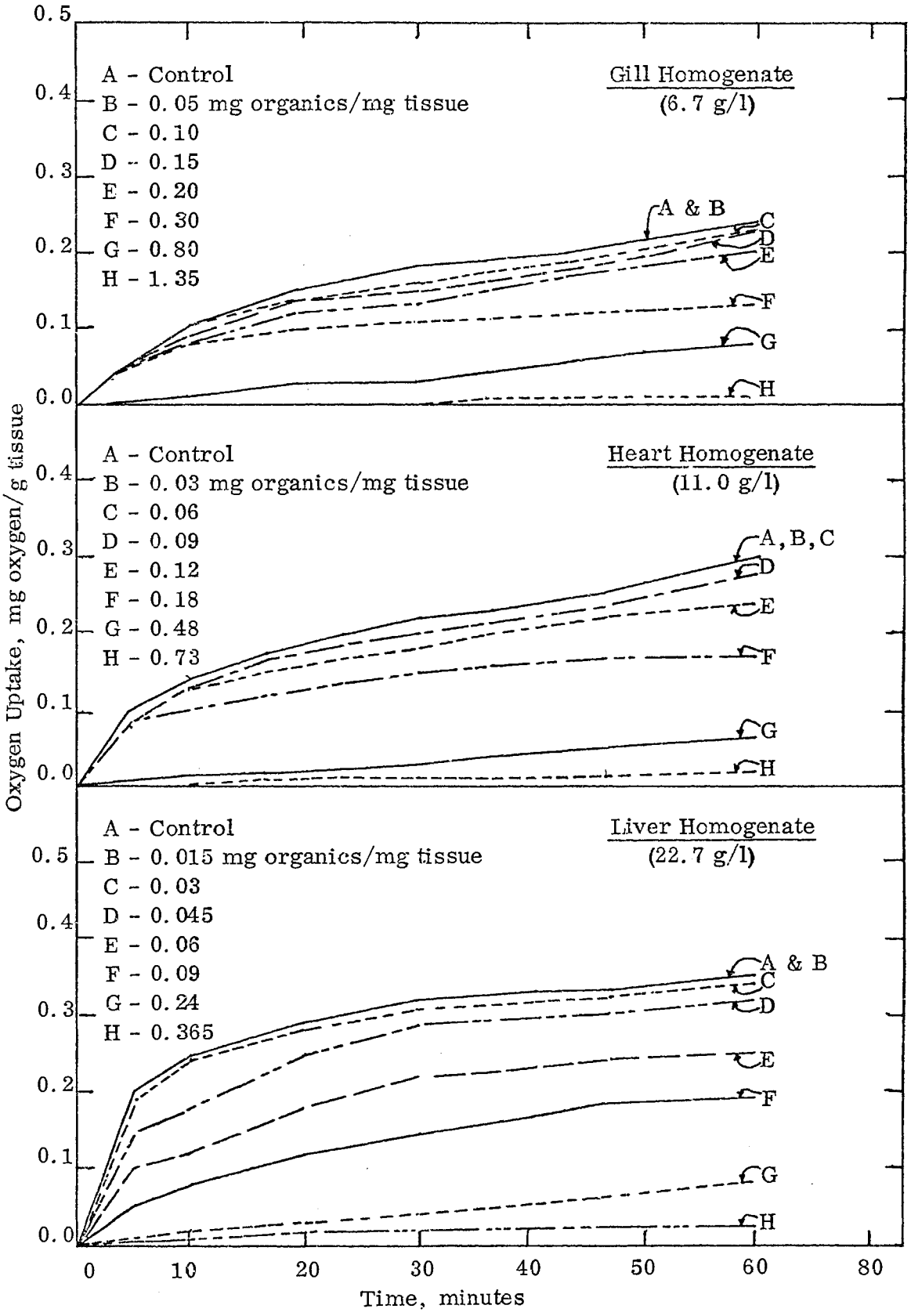

Figure 6

Effect of Treated Missouri River Water CCE On
Trout Homogenate Respiratory Enzyme Activity

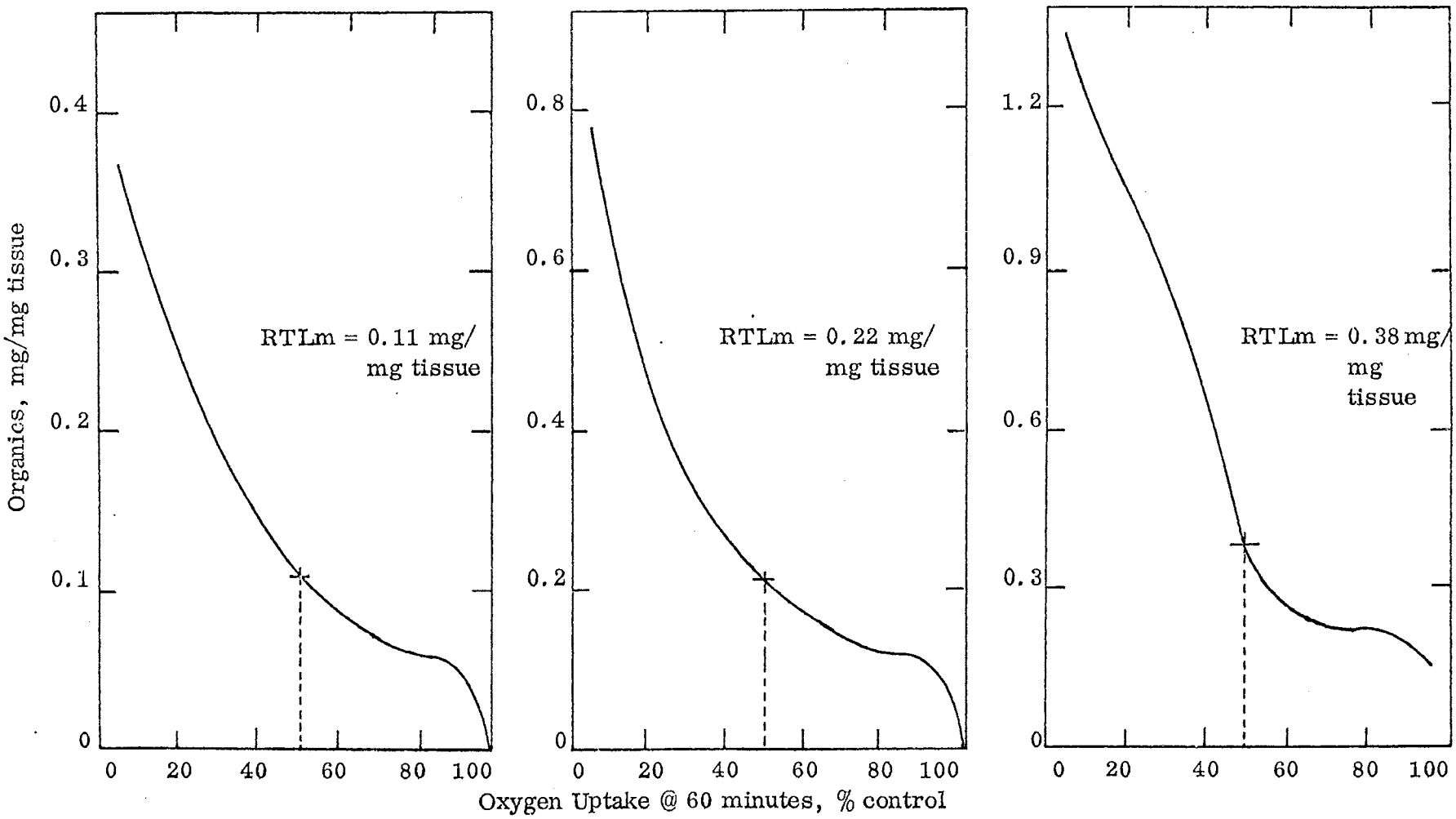

Figure 7

Median Respiratory Tolerance Limit of Trout
Tissue Homogenate Exposed to Treated Missouri River Water CCE

The results of the tissue studies definitely indicated that while the surface water organics inhibited respiratory enzyme activity, the subsurface water extracts and the pesticides exerted no inhibition to the activity of trout tissue homogenates.

2. Oxygen Transfer Studies

In an attempt to evaluate further the mode of action of the trace organics, the effect of these materials on the transfer of oxygen across a semipermeable membrane was investigated using an especially developed model. This model consisted of an air tight cylindrically shaped reaction vessel equipped with facilities for measuring and purging dissolved oxygen. The detailed dimensions of the reaction vessel are shown in Figure 8, and the overall experimental arrangement is pictured in Figure 9. Oxygen transfer was evaluated using either a silicone membrane* reported by the supplier to be specific for the transfer of carbon dioxide and oxygen which was formed into a cylindrical shape and sealed with silicone cement to make it water tight or a cylindrical cellulose dialysis membrane.** The membrane cylinder was placed in the model unit and filled with deionized water; a small (1/16 inch diameter) purging tube connected to a nitrogen supply was inserted in the cylinder. The test solution was placed in the unit and was mechanically stirred. The dissolved oxygen and temperature probes of a Precision galvanic cell dissolved oxygen analyzer*** were also inserted in the unit.

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*Catalog No. 40,824, a product of Edmond Scientific, Inc., Barrington, N.J.
**Catalog No. 8-667-4, a product of the Fisher Scientific Company, St. Louis, Mo.
***Catalog No. 68850, a product of the Precision Scientific Co., Chicago, Ill.

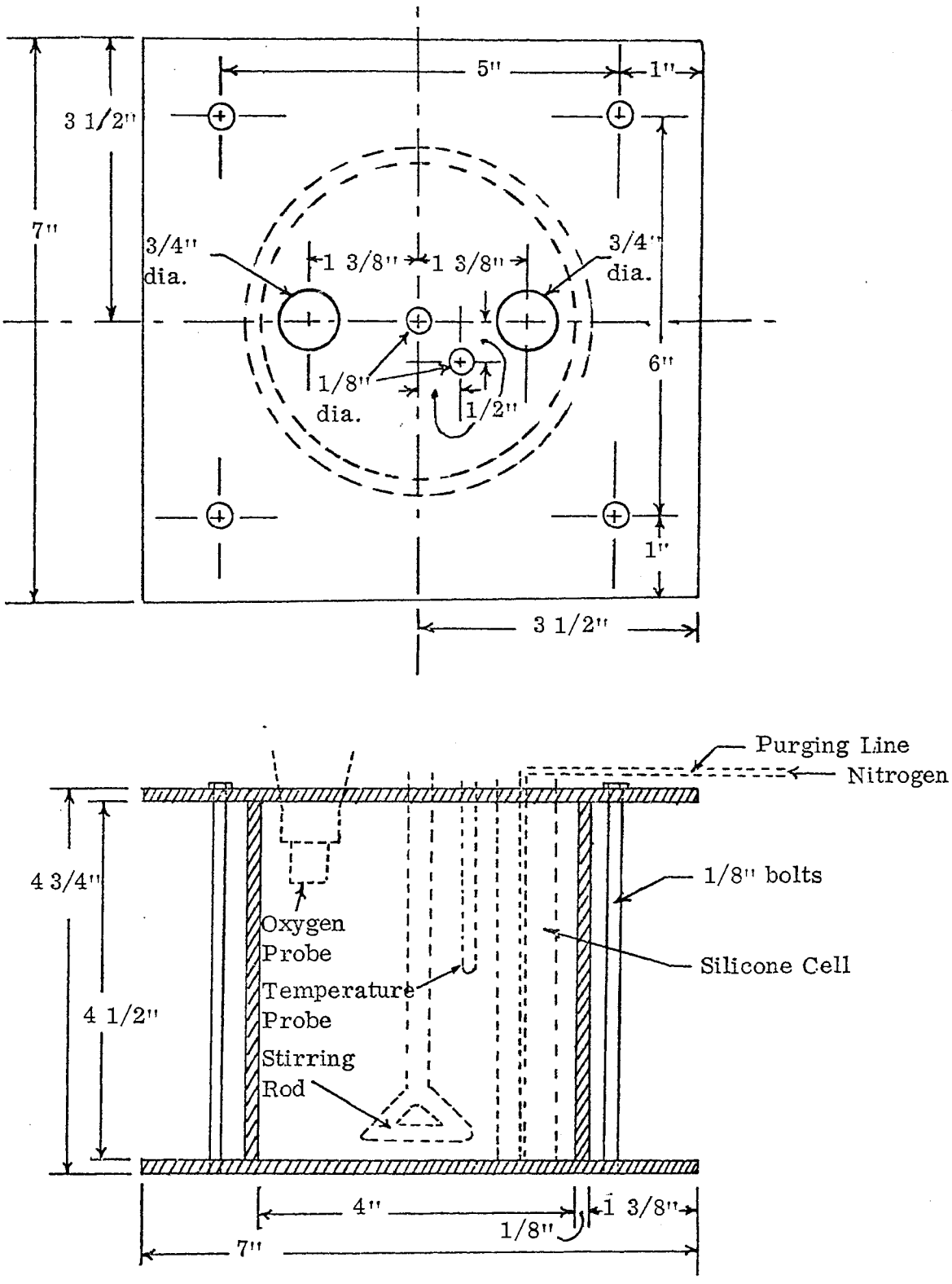

Figure 8

Model for Oxygen Transfer Studies

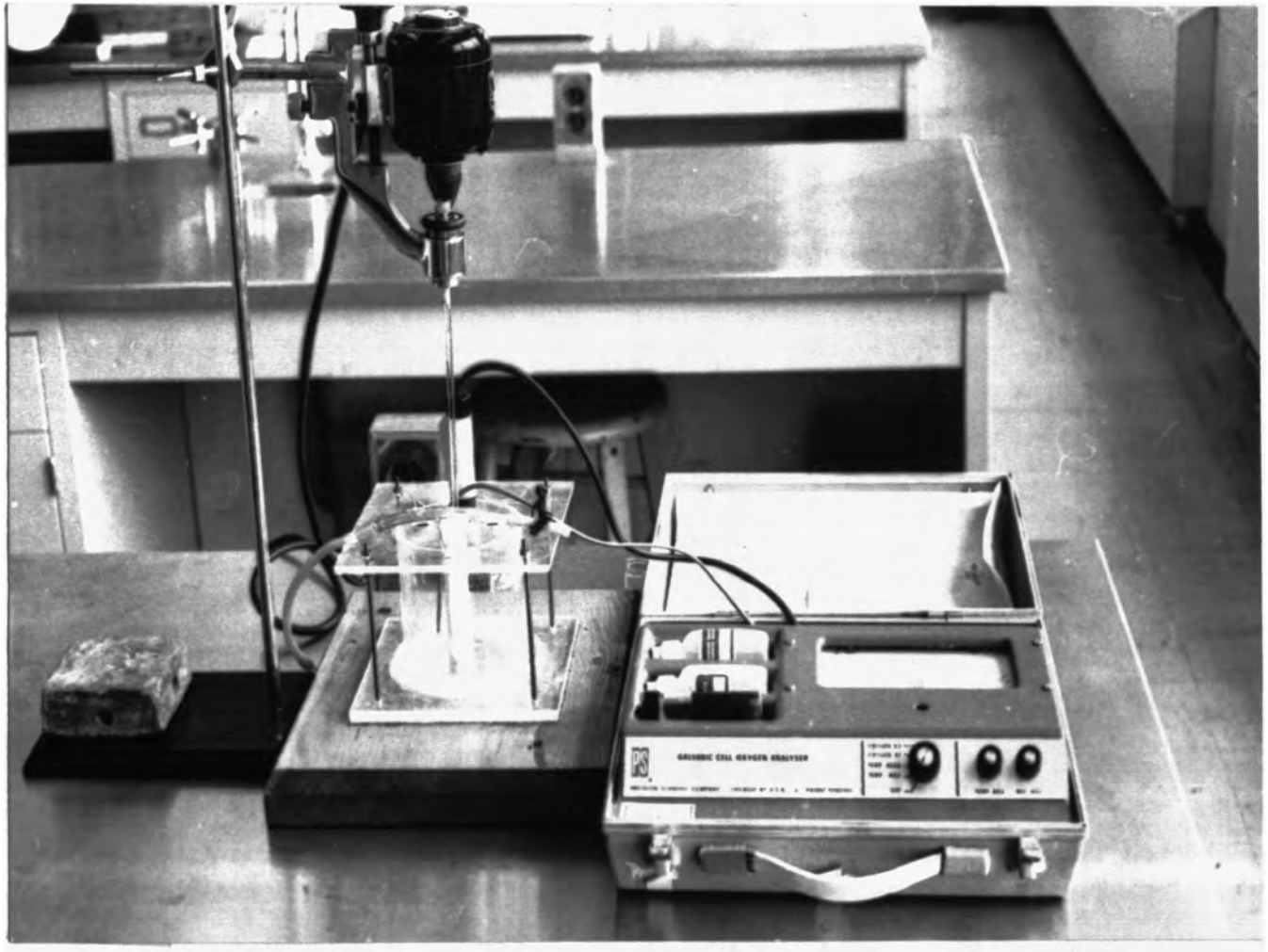

Figure 9

Experimental Arrangement for Oxygen Transfer Studies

The initial dissolved oxygen concentration in the test solution was measured, and purging at a uniform rate was started; as the oxygen inside the membrane cylinder was removed, a dissolved oxygen concentration gradient was set up between the test solution and the water inside the cylinder causing oxygen to flow across the membrane from the test solution to the water. The test was continued until either the test solution had become deoxygenated or the rate of deoxygenation became very small. The dissolved oxygen analyzer was calibrated daily using the alkali-azide modification of the Winkler dissolved oxygen determination method (16, p. 406).

The transfer efficiency of the two membranes was evaluated using tap water as the test solution and determining the time required to purge completely the test solution. Oxygen transfer efficiency curves for these membranes are shown in Figure 10, and the corresponding data are given in Table C-1, Appendix C. Oxygen transfer efficiency was defined as the amount of dissolved oxygen removed after a given time from the test solution by transfer across the membrane divided by the initial dissolved oxygen concentration. The silicone membrane permitted faster deoxygenation of the test solution and was employed in the oxygen transfer studies.

The effect of the subsurface and surface water organic micropollutants on the oxygen transfer across the membrane was determined. Tap water was used before and after each test to evaluate the condition of the membrane. Both individual CCE and CAE materials from Meramec Spring Run #1 Unit #1, Run #2 Units #1, 2, and 3, and CCE and CAE combined at the natural ratios were studied. The results are presented in Table C-2, Appendix C. The

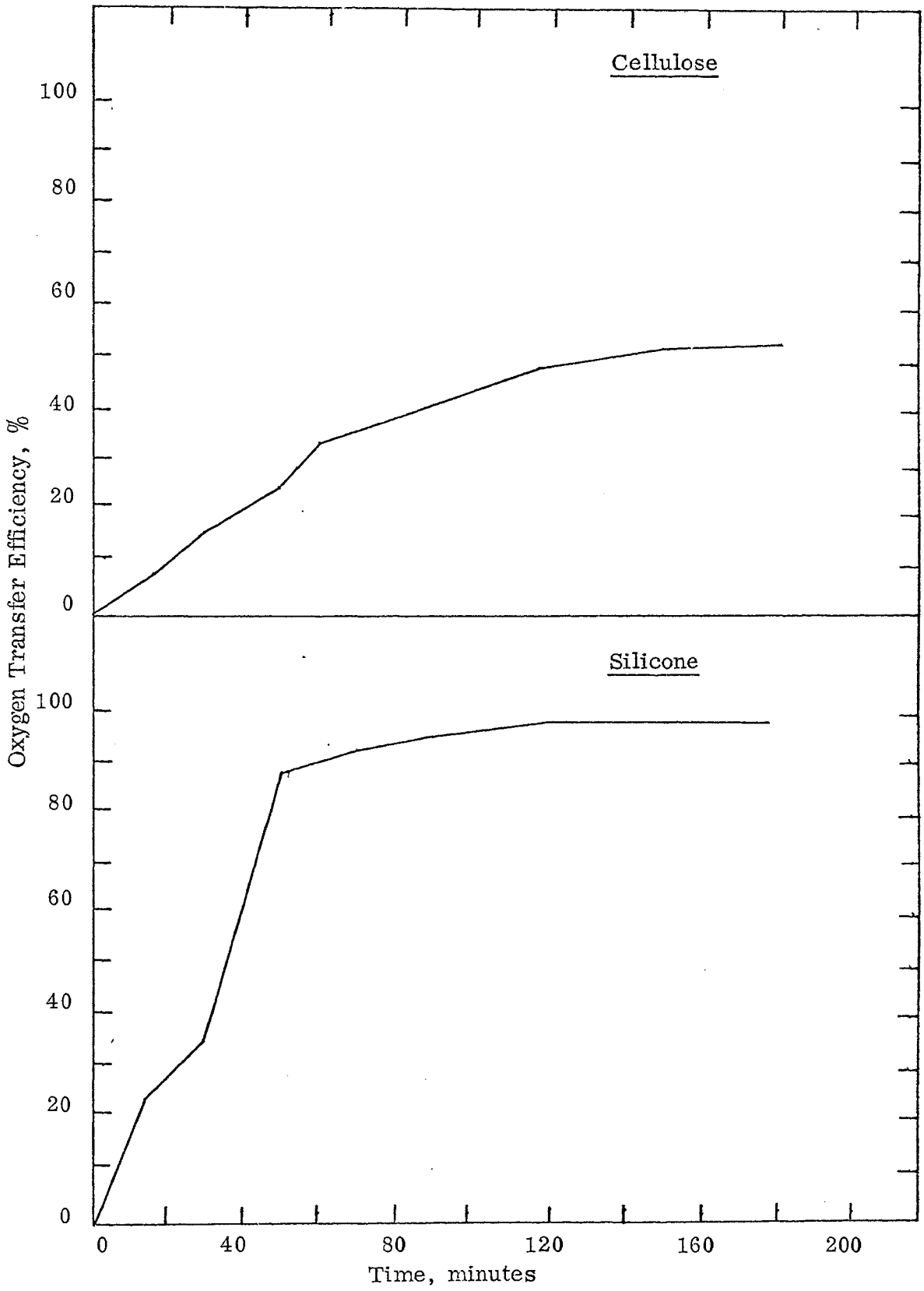

Figure 10

Oxygen Transfer Efficiency Curves For
Silicone and Cellulose Membranes

individual CCE and CAE organics exerted no effect on the oxygen transfer across the membrane, while the combined CCE and CAE, except for those recovered in Run #2 Unit #3, appeared to clog the membrane and inhibit oxygen transfer. Oxygen transfer efficiency curves for the combined subsurface water CCE and CAE are shown in Figure 11.

The effect of the test solution concentration is shown in Figure 12 using Run #2 Unit #1 combined extracts. At a concentration of 65 mg/l, or one-half the 24 hour TLm for trout, the oxygen transfer efficiency was reduced to 40 per cent, while at a concentration equal to or greater than the 24 hour TLm (130 mg/l), the efficiency was reduced to 28 per cent.

The cumulative effect of the trace organics on oxygen transfer was evaluated by sequentially exposing a membrane to concentrations of Meramec Spring Run #2 Unit #1 combined extracts equal to 10, 30, and 50 per cent of the 24 hour TLm value. The membrane was evaluated before and after each test solution with tap water to determine its condition. The results of these studies are given in Table C-3, Appendix C, and are plotted in Figure 13. Differences in transfer efficiency of the membrane were observed in tap water studies performed before and after each test solution experiment; as the membrane was sequentially exposed to higher concentrations of organics, its oxygen transfer efficiency in tap water was significantly decreased. This would indicate that the organic materials were accumulating on the membrane and were bound securely to it. A comparison of the transfer efficiency at the end of 180 minutes of the membrane after it had been exposed to all three concentrations (a cumulative total of 118 mg/l, an efficiency of 28 per cent) and a membrane

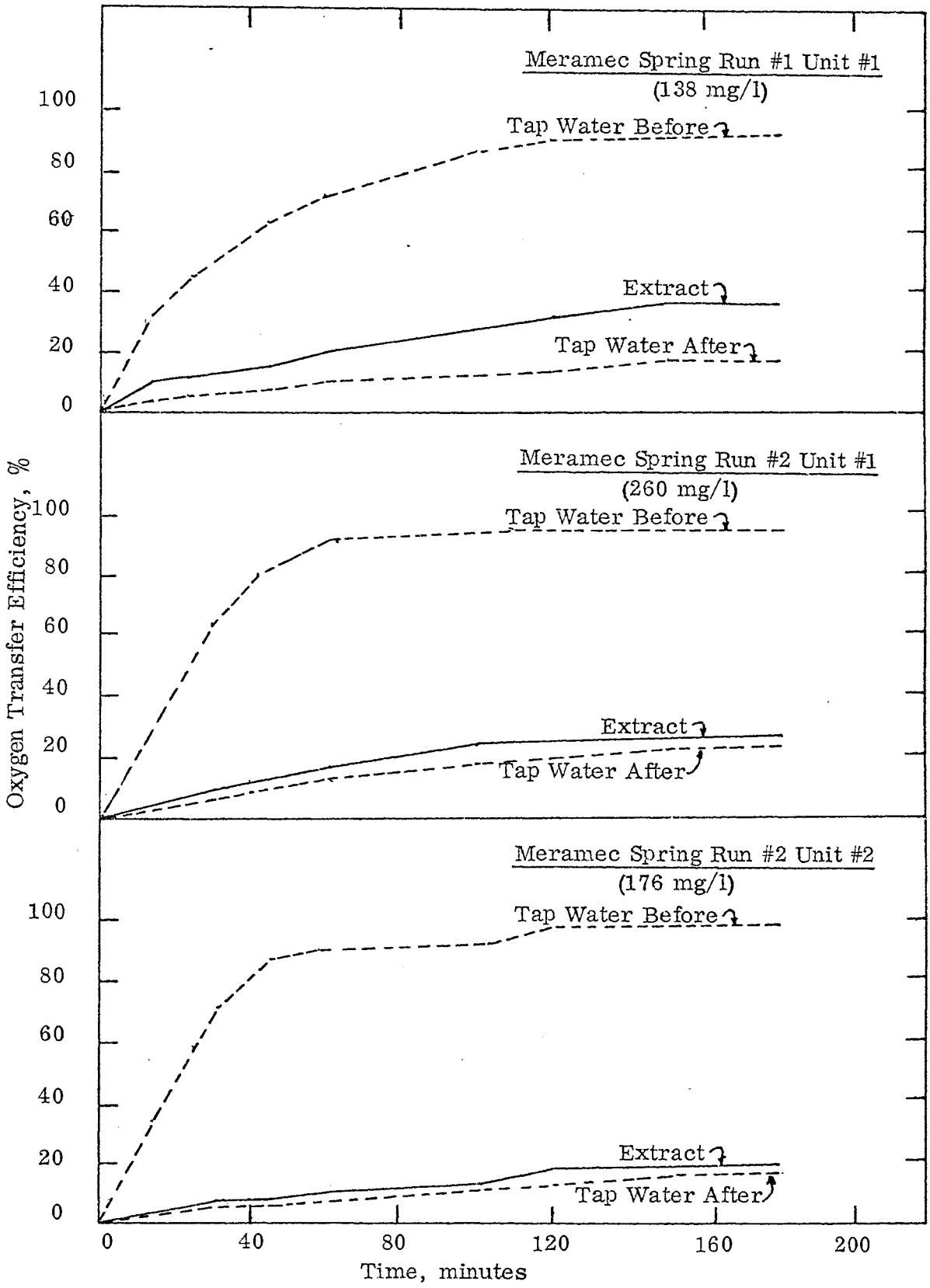

Figure 11

Effect of Subsurface Water CCE and CAE
On Oxygen Transfer Efficiency

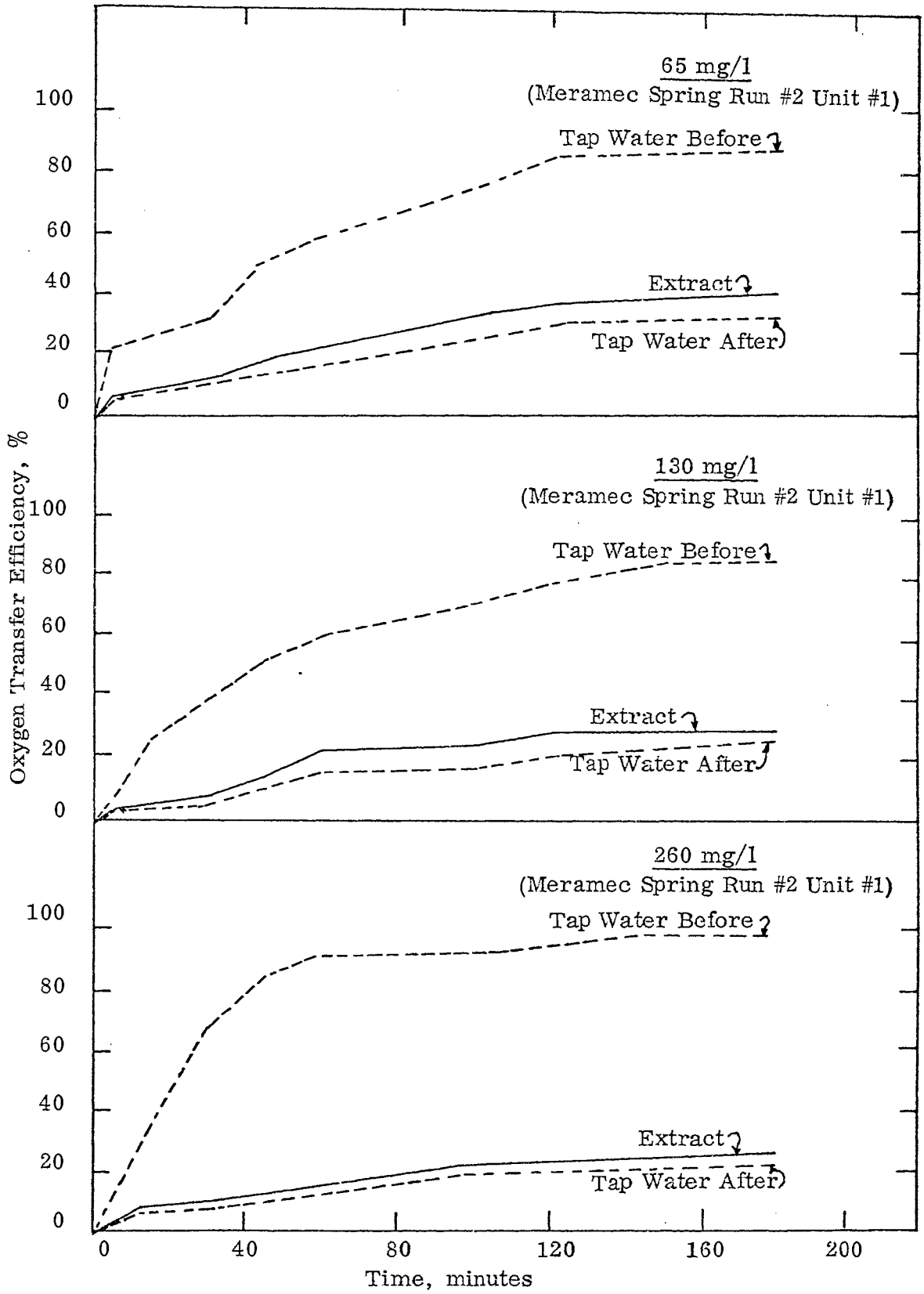

Figure 12

Effect of Subsurface Water CCE and CAE Concentration
On Oxygen Transfer Efficiency

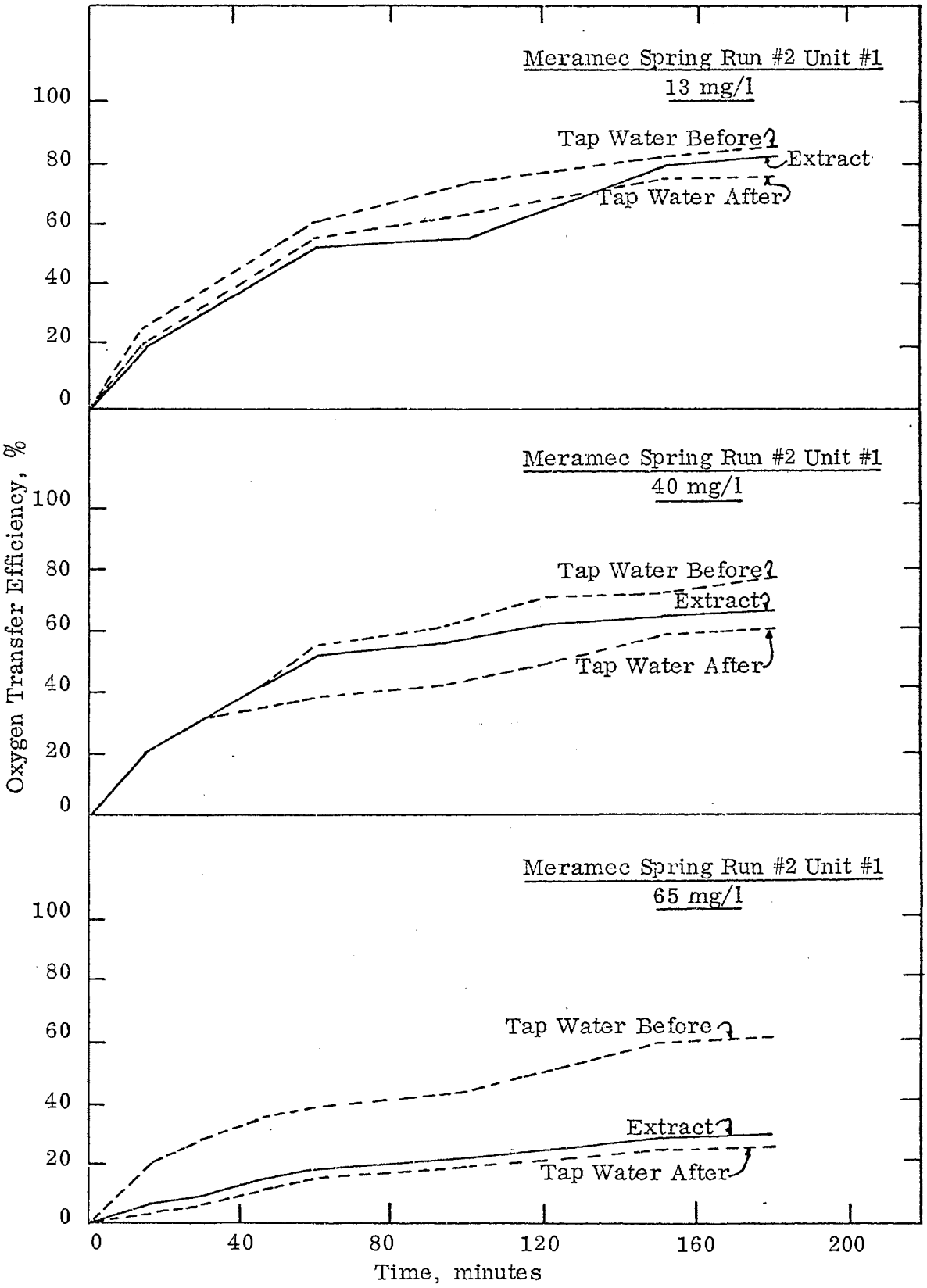

Figure 13

Cumulative Effect of Subsurface Water CCE and CAE
On Oxygen Transfer Efficiency

exposed directly to an approximately equal concentration (130 mg/l, an efficiency of 28 per cent, see Figure 12) indicates further the accumulation of the organics on the membrane.

Membranes which had been clogged by the subsurface water trace organics were subjected to infrared analysis in an attempt to identify the accumulated material. The infrared spectra from these studies are presented in Figure 14. No differences were detected between the unexposed and the clogged membranes; the small amount of material accumulated on the membrane was probably beyond the detection limit of the instrument.

Contrary to the combined subsurface water CCE and CAE extracts, the surface water CCE did not affect the transfer efficiency of the membrane; oxygen transfer efficiency data are presented in Table C-4, Appendix C.

3. Mode of Action

An evaluation of the results of the oxygen transfer and enzyme studies would indicate that the mode of action of the surface and subsurface organics was different. The treated Missouri River water CCE disrupted the internal enzymatic utilization of oxygen. On the other hand, the combined CCE and CAE from Meramec Spring Run #1 Unit #1 and Run #2 Units #1 and 2 blocked the physical transfer of oxygen across the gills but did not affect the respiratory enzyme activity even at the high concentrations evaluated. It should be pointed out that the extracts from Run #2 Unit #3 of the spring, which were not toxic to fish at the concentrations evaluated, did not affect either the oxygen transfer or the respiratory enzyme activity. The oxygen transfer studies with tap water before and after a test solution demonstrated that the organics which

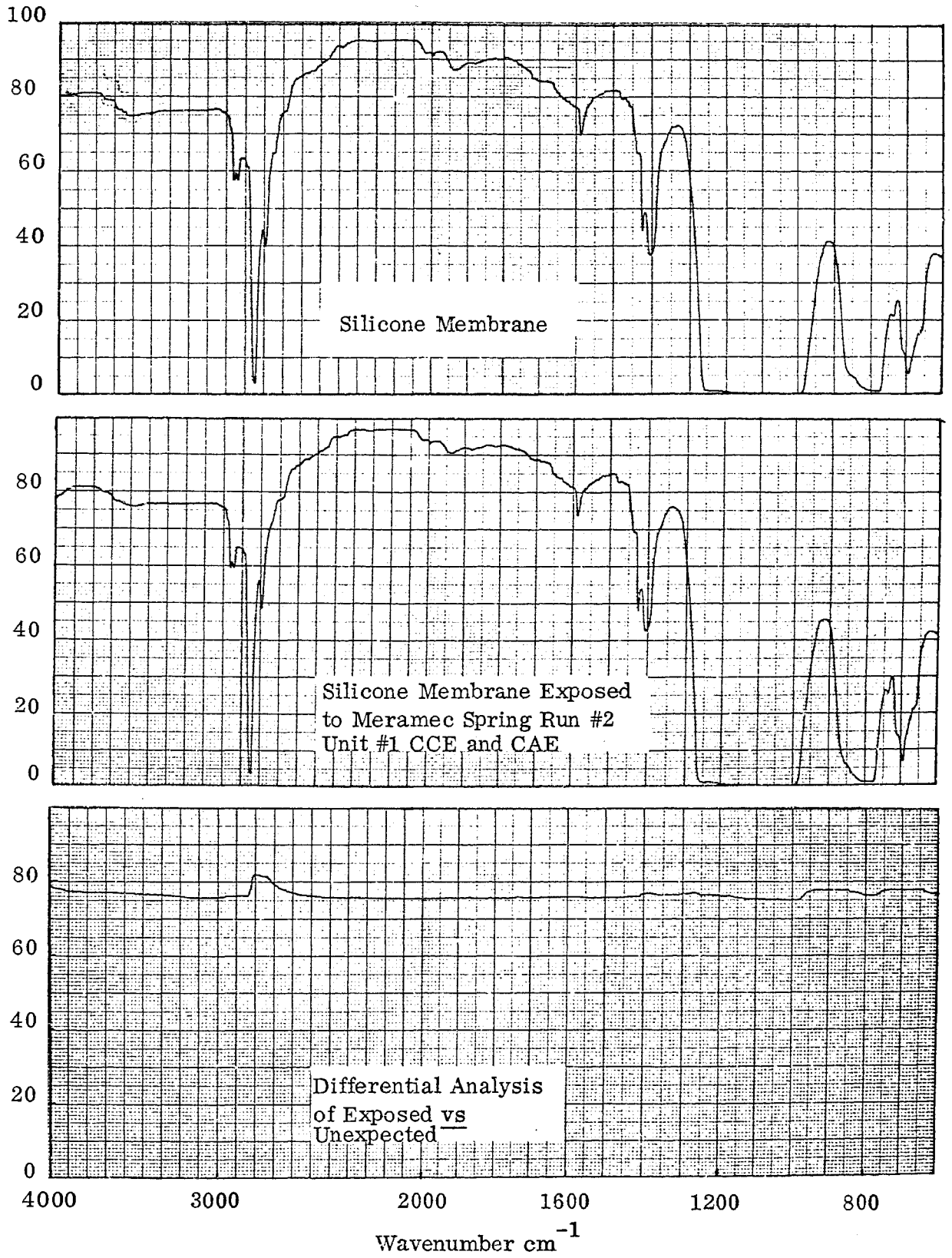

Figure 14

Infrared Spectra of a Silicone Membrane
And a Silicone Membrane Exposed to Subsurface Water CCE and CAE

clogged the membrane were tightly bound to it; the transfer efficiency of the membrane exposed to tap water after being exposed to a test solution was lower (except for the 13 mg/l study) than the transfer efficiency of the membrane exposed to the test solution. The pesticides exerted no inhibition on the respiratory enzyme activity; sevin (67) and malathion (68, p. 742) have been reported to inhibit brain cholinesterase activity; however, this aspect was not evaluated in this study.

C. LONG-TERM STUDIES

The acute toxicity studies illustrated that some of the trace organics were toxic to fish at high concentrations over a relatively short period of time. Realistically, some of the higher concentrations investigated may never be reached under normal conditions; however, the long-term effects of these materials at low concentrations over an extended period of time could be significant. Consequently, studies were performed with selected trace organics to evaluate their long-term toxic effects and to develop a procedure whereby long-term median tolerance levels, toxicant levels at which only 50 per cent of the fish would be killed, could be estimated from short-term data.

1. Long-Term Toxicity Studies

Because the limited quantity of test material available and the time involved in recovering the organics from water ruled out continuous flow studies which have been used by other investigators (47, 48, 69, 70), toxicity studies were conducted using a batch-type long-term bioassay. The procedure employed consisted of exposing the fish to the test solution under static conditions for a period of 5 days, removing the fish, and placing them in a recovery solution

for 5 days during which time they were fed daily. The recovery solution consisted of either fresh water or water containing one-tenth the test concentration of organics. At the end of the recovery period, the fish were placed in fresh test solution for another 5 day period, and the procedure was continued until at least 50 per cent of the test animals had been killed.

The exposure and recovery periods were determined using a 60 mg/l test concentration of combined CCE and CAE (Meramec Spring Run #2 Unit #1) organics in a series of experiments with trout. Control systems which did not contain any organics were also used. To determine the optimum exposure time, the fish were exposed to the organics for periods ranging from 3 to 8 days, placed in a fresh water recovery solution for 3 days during which time they were fed daily, and the cycle was repeated until the control fish died or the test fish exhibited erratic rates of death. To determine the optimum recovery time, the same general procedure was employed except that the fish were exposed to the organics for 5 days and placed in the recovery solution for periods ranging from 1 to 10 days. The results of these studies are given in Table 15. It was found that after 5 days of exposure, the test fish in both the control and the test solutions exhibited erratic deaths. It was, therefore, concluded that 5 days was the maximum length of time the fish could exist without feeding and remain in an acceptable physical condition for the bioassay studies. It was also found that for recovery periods of less than 5 days the fish in the control unit did not survive more than 15 days of accumulated exposure time, while the fish exposed to the toxicant exhibited erratic death rates indicating that they had not completely recovered from the lack of feeding. For recovery periods greater

Table 15

Evaluation of Exposure and Recovery Periods
For Long-Term Toxicity Studies

Test		Exposure or Recovery Time, days	Accumulated Exposure Time for Given Number of Deaths, days											
			1	2	3	4	5	6	1	2	3	4	5	6
Number	Type		Control						Organics*					
1	Exposure**	3	--	--	--	--	--	--	6	7	8	8	8	9
2		4	--	--	--	--	--	--	6	7	7	8	8	9
3		5	--	--	--	--	--	--	7	7	8	8	9	9
4		6	8	10	--	--	--	--	6	6	7	7	7	7
5		8	6	7	10	15	--	--	5	6	6	7	7	7
6	Recovery***	1	6	6	7	7	8	10	6	6	7	7	7	7
7		2	6	6	7	8	8	10	6	6	7	7	7	7
8		4	10	11	12	12	13	14	6	6	7	7	7	7
9		5	--	--	--	--	--	--	8	8	9	9	10	10
10		8	--	--	--	--	--	--	8	8	8	9	9	10
11		10	--	--	--	--	--	--	8	9	9	10	10	10

*Meramec Spring Run #2 Unit #1 combined CCE and CAE, 60 mg/l.
**A 3 day recovery period was used.
***A 5 day exposure period was used.

than 5 days, the control fish survived at least 30 days accumulated exposure time, while all the test fish died between 8 and 9 days of accumulated exposure time. On the basis of these findings, both the exposure and recovery times were set at 5 days each.

The results of the long-term toxicity investigations are presented in Table 16. The total accumulated test time indicated was the total length of time the fish had been exposed to the toxicants and does not include the corresponding recovery time. In addition to the surface and subsurface water organic micropollutants, malathion was also used in these studies to provide data with a known material for the reevaluation of the procedure, if necessary. The results presented in Table 16 have been duplicated in parallel runs. For comparative purposes, the 96-hour TLm value for each toxicant is also presented in this table. When the exposure time was lengthened, the trace organics and malathion were toxic to the test fish at concentrations well below the acute toxicity levels. A comparison of the time required for 50 per cent kill at a given concentration level in studies using recovery solution consisting of fresh water and studies using a 10 per cent concentration of the test solution further indicated that the organic micropollutants did have a cumulative effect and that there was a buildup of toxicant in the fish.

Extreme difficulty with disease in the test fish, both trout and minnows, was encountered. Although every effort was made to secure healthy fish for the tests and the fish were held in the laboratory under static conditions for at least 10 days before use and regularly treated with antibiotic and formalin baths, some of the control fish exhibited signs of being diseased during the long-term

Table 16

Long-Term Toxicity of Organic Micropollutants from Missouri Waters

Test Material	Test Fish (Length, cm/Weight, g)	Total Accumulated Test Time* for 50% Kill, days								96 hour TLm Value mg/l
		Test Material Concentration, mg/l								Control
		10		7.5		4.2		1.0		
		0**	1.0**	0	0.75	0	0.42	0	0.10	
Meramec Spring Run #2 Unit #2 CCE & CAE	Trout (8.2/11.0)	30	20	37	24	48	29	>54	>54	>54***	61-155†
Missouri River at St. Louis CCE	Trout (5.3/2.2)	10	5	13	11	19	13	>19	>19	>19‡	28
		24		13.5		5.6				Control
		0	2.4	0	1.35	0	0.56			
Meramec Spring Run #2 Unit #2 CCE & CAE	Red Shiners (4.7/1.5)	30	20	39	30	>65	45			>65‡	148
		0.0010		0.00075		0.00056		0.00024		Control
		0	0.00010	0	0.000075	0	0.000056	0	0.000024	
Malathion	Trout (6.0/3.8)	7	5	15	8	>17	10	>17	>17	>19	0.0028

*Five day exposure and recovery periods were used.

**Test material concentration in recovery water, mg/l.

***Fish died because of loss of temperature control.

†Values for trout having 10.5/16.0 and 5.0/2.6, length/weight, respectively.

‡Fish showed signs of disease; test discontinued.

studies. A test was halted when one of the control fish had died, because it was felt that continuing it would have given erroneous data. The static conditions employed in the long-term studies may have been responsible for this occurrence of disease, which may not have happened if continuous flow studies had been used. However, due to the limited quantity of test materials available for evaluating the toxicity of micropollutants, a continuous flow study was not practical. Difficulty was also encountered in one of the trout studies with loss of temperature control in the test room which resulted in the death of all the test fish.

2. Prediction of Long-Term Toxicity Levels

Because of the length of time required to evaluate long-term toxic effects and the problems encountered with the extended batch-type bioassay, the development of a method for predicting long-term toxic levels from short-term tests was attempted. Before a mathematical relationship between short-term data and expected long-term values could be developed, a correlation between toxicant concentration and some parameter related to individual fish characteristics was necessary. This was shown by the acute toxicity data (see Table 13, p. 53) where the fish size, within the same species, had a marked effect on the toxicity of the subsurface water organics; the smaller fish were more resistant to the test material.

The mode of action studies revealed that the subsurface water organics were affecting oxygen transfer across the gills; consequently, the surface area of the gills had to be taken into consideration when relating toxic concentration to fish characteristics. In addition, since the amount of oxygen transported

across the gills was dependent on the demand for oxygen by the fish, which in turn was dependent on the size of the fish, a factor relating body activity to toxic concentration was also required. The condition factor, K, defined by Lagler, et al. (71, p. 173) as the weight of the fish divided by its length cubed, was employed as a parameter representing the size and, indirectly, the oxygen demand of the test fish. Various combinations of the toxicant concentration, gill surface area, and condition factor were evaluated in an attempt to find a relationship between toxic concentration and fish characteristics. Acute toxicity data for trout exposed to Meramec Spring Run #2 Unit #2 combined CCE and CAE organics (Table 13) were employed to develop this relationship; the acute toxicity of these materials to two different sizes of trout varied inversely with the gill surface area (GSA) and directly with the condition factor. Of the many combinations of concentration, GSA, and K evaluated, the following relationship was found to normalize the characteristics of the test fish and the TLm concentration:

$$y_t = C_t \cdot \left[\frac{GSA}{K}\right]^{1.25} \qquad \text{(Equation 1)}$$

where: y_t = toxicity factor relating the toxicant TLm value at time t with test fish characteristics

C_t = TLm value at time t, mg/l

GSA = gill surface area, mm^2

K = condition factor, g/cm^3

Toxicity factors (y_t) for the subsurface water organics were calculated on the basis of this equation using the acute (Table 13) and long-term (Table 16) TLm values and the physical characteristics of the test fish (Table 14), and are

presented in Table 17 (experimental values). When the toxicity factor was plotted against total accumulated test time in days, a line which had a constant initial slope but gradually curved and became asymptotic to the time axis resulted; this is shown in Figure 15 (experimental curve) for trout exposed to the Meramec Spring Run #2 Unit #2 CCE and CAE organics. A curve of this general form can be described by the following general equation (72, p. 12-11):

$$f_t = f_c + (f_o - f_c)\, e^{-bt} \qquad \text{(Equation 2)}$$

where: f_t = value of the function at time t

f_c = value of f when the curve becomes asymptotic to the time axis

f_o = value of f at time zero

b = slope of the curve

In terms of the toxicity factor, this equation becomes

$$y_t = y_c + (y_o - y_c)\, e^{-bt} \qquad \text{(Equation 3)}$$

with y_o and y_c the toxicity factors for the immediate and long-term (eventual) TLm values, respectively. The constants for Equation 3 were determined from the acute toxicity data (Table 13). When the toxicity factor was plotted against time on semilogarithmic paper (see Figure D-1, Appendix D) the acute toxicity values formed a straight line. This line could be extended to the toxicity factor axis (t = 0), and the value of y_o obtained. It should be noted that this value for the spring Run #2 Unit #2 extracts was very close to the toxicity factor computed on the basis of the concentration which killed one-half of the test fish within 2 hours (determined from the preliminary toxicity studies). The value of y_c was

Table 17

Toxicity Factors for Test Fish Exposed to Subsurface Water CCE and CAE

Time days	Test Material	Time mg/l	Test Fish Characteristics					Toxicity Factor, $y_t \times 10^{-7}$	
			Type of Fish	Length cm	Weight g	GSA mm^2	K x 10^{-2} g/cm^3	Experimental*	Theoretical**
1	Meramec Spring Run #2 Unit #2 CCE & CAE (1/3.35)	201	Trout	5.0	2.6	2,400	2.08	2.48	2.50
		88		10.5	16.0	3,100	1.38	2.48	
2		186		5.0	2.6	2,400	2.08	2.24	2.29
		75		10.5	16.0	3,100	1.38	2.24	
4		155		5.0	2.6	2,400	2.08	1.79	1.87
		61		10.5	16.0	3,100	1.38	1.79	
5		130		5.0	2.6	2,400	2.38	1.56	1.70
		56		10.5	16.0	3,100	1.38	1.56	
30		10		8.2	11.0	4,300	1.99	0.26	0.22
37		7.5		8.2	11.0	4,300	1.99	0.20	0.17
48		4.2		8.2	11.0	4,300	1.99	0.11	0.12
60		--		--	--	--	--	--	0.11
70		--		--	--	--	--	--	0.10
1		195	Red Shiners	5.7	2.3	2,320	1.24	78	78
2		170		5.7	2.3	2,320	1.24	68	70
4		148		5.7	2.3	2,320	1.24	59	58
5		120		5.7	2.3	2,320	1.24	48	52
30		24		4.7	1.5	2,780	1.45	9.6	10
39		13		4.7	1.5	2,780	1.45	5.4	5.4
50		--		--	--	--	--	--	4.5
60		--		--	--	--	--	--	4.2
70		--		--	--	--	--	--	4.1
80		--		--	--	--	--	--	4.0

*Calculated using Equation 1.
**Calculated using Equation 4.

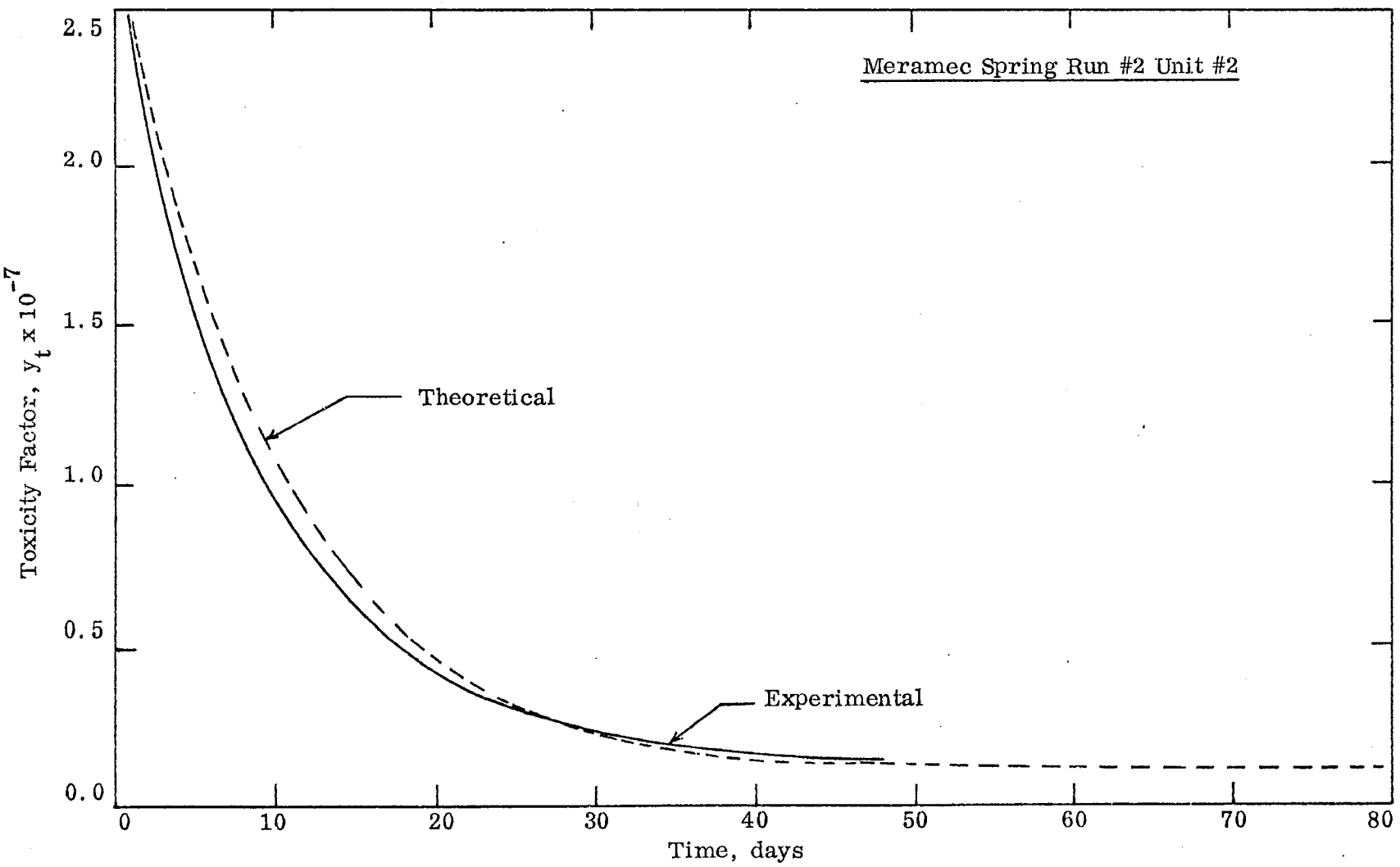

Figure 15

Toxicity Curves for Trout
Exposed to Subsurface Water CCE and CAE

determined by trial and error solution of Equation 3 for two different times. This procedure is outlined with an example in Appendix D. After the values of y_o and y_c had been determined, the slope b was computed.

The toxicity equation derived for the Meramec Spring Run #2 Unit #2 extracts and trout (see Appendix D) was as follows:

$$y_t = 0.10 \times 10^7 + (2.7 \times 10^7)\ e^{-0.11t} \qquad \text{(Equation 4)}$$

Theoretical y_t values determined on the basis of this equation are given in Table 17 and are plotted in Figure 15. As may be seen from this figure, the experimental and theoretical curves were almost identical.

If the mathematical model were correct, it should have been valid for other types of fish exposed to the same test material. The acute toxicity values (Table 13) for red shiners exposed to Meramec Spring Run #2 Unit #2 combined organics were converted to toxicity factors using Equation 1 and are presented in Table 17. A toxicity equation was developed utilizing the procedure described previously and the constants given in Table D-1, Appendix D; this equation was as follows:

$$y_t = 4.0 \times 10^7 + (82.0 \times 10^7)\ e^{-0.11t} \qquad \text{(Equation 5)}$$

The y_t values computed on the basis of this equation are shown in Table 17 and Figure 16 together with the values obtained from the experimental studies. As in the case of trout, the theoretical values closely approximated the experimental.

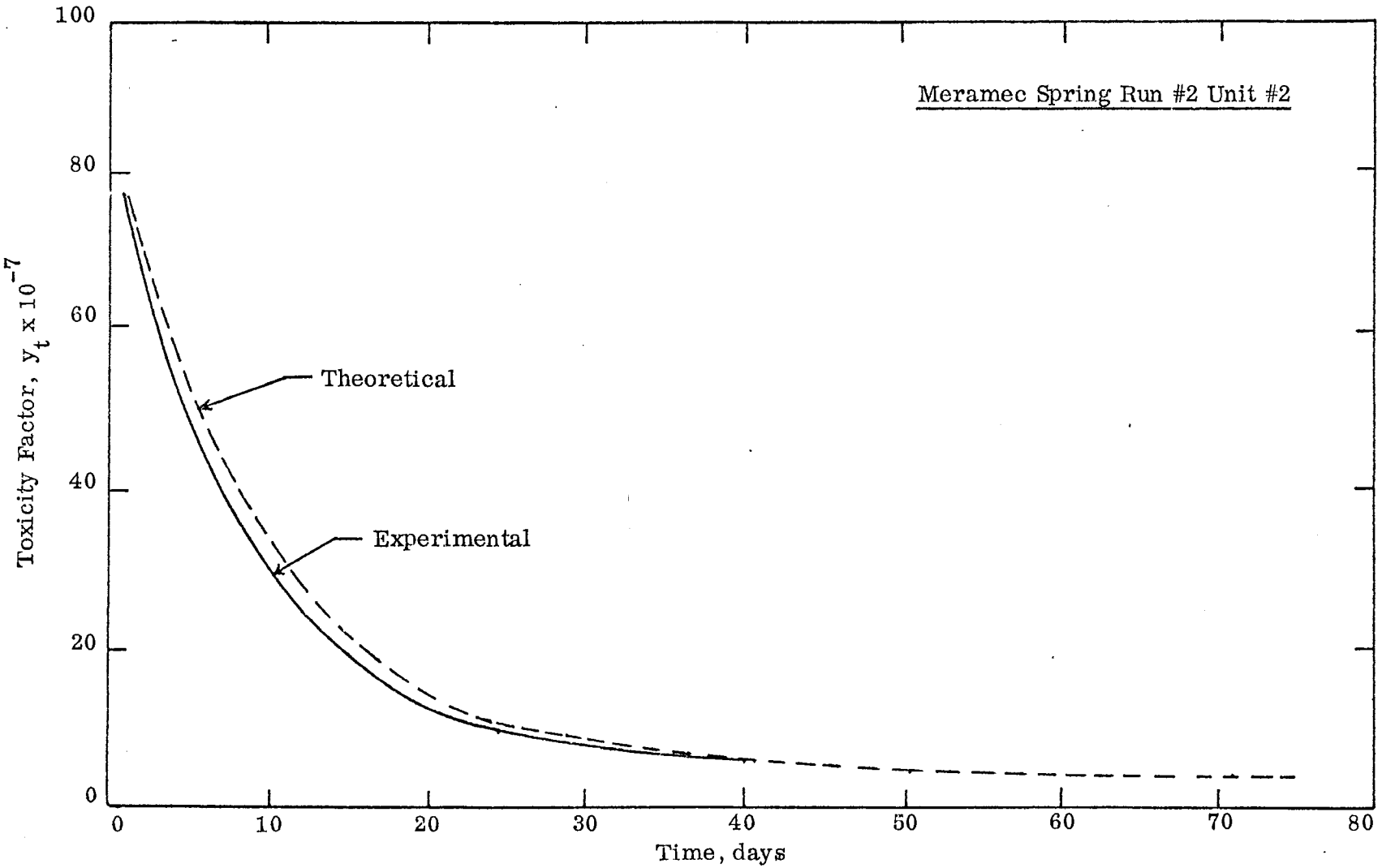

Figure 16

Toxicity Curves for Red Shiners
Exposed to Subsurface Water CCE and CAE

The mathematical model given by Equation 3 appeared to hold for the subsurface water organics when the toxicity factor was correctly defined. Since the mode of action of the surface water extracts and malathion was different from that of the subsurface materials, the toxicity factor had to be redefined for these toxicants. Contrary to the subsurface water organics which physically blocked the transfer of oxygen across the gills, the surface water organics inhibited the internal enzymatic utilization of oxygen. Consequently, the gill surface area was not considered applicable, and the toxicity factor was redefined as a relationship between TLm concentration (C_t) and the condition factor (K). Toxicity factors were calculated for different combinations of C_t and K using the acute toxicity values (Table 13) for trout exposed to treated Missouri River water CCE. Corresponding toxicity equations were derived for these toxicity factors employing the procedure described in Appendix D for subsurface water organics, and were used to estimate the toxic concentrations at the end of 10, 13, and 19 days at which times experimental values were available (Table 16). The estimated values were then compared to the experimental values to determine which of these relationships was valid for both the acute and long-term studies. It was concluded that the relationship

$$y_t = C_t/K \qquad \text{(Equation 6)}$$

best correlated the acute and long-term data. Mode of action studies with malathion indicated that it did not affect either the internal respiratory enzyme activity or the physical transfer of oxygen across the gills; on this basis, Equation 6 was also used to define the toxicity factor for this pesticide.

The toxicity factors for surface water CCE and malathion were calculated using Equation 6 on the basis of the corresponding acute (Table 13) and long-term (Table 16) toxicities and the physical characteristics of the fish (Table 14), and the values obtained are presented in Table 18. Using the procedure outlined in Appendix D, corresponding toxicity equations for Missouri River water CCE

$$y_t = 0.30 \times 10^{2} + (20.7 \times 10^{2})\ e^{-0.11t} \qquad \text{(Equation 7)}$$

and for malathion

$$y_t = 0.10 \times 10^{-2} + (46.7 \times 10^{-2})\ e^{-0.23t} \qquad \text{(Equation 8)}$$

were developed. Theoretical toxicity factor values for these two toxicants were calculated and are presented in Table 18 and plotted in Figures 17 and 18. It can be seen from the data presented that the correlation between the experimental and theoretical values was good, although perhaps not so close as the correlation obtained with the subsurface water organics.

To evaluate further the applicability of the mathematical model for predicting long-term toxic levels, this method was applied to acute and long-term data reported by other investigators. Henderson, et al. (69) studied the acute and long-term effects of acrylonitrile on flathead minnows using continuous flow experiments and found, by means of visual observation of the affected fish, that the gills appeared normal while some internal hemorrhaging was present. Based on their observations, the toxicity factor could be defined as C_t/K. The acute toxicity data reported by Henderson and his coworkers were used to calculate

Table 18

Toxicity Factors for Test Fish Exposed to a Surface Water CCE and a Pesticide

Time days	Test Material	TLm mg/l	Test Fish Characteristics				Toxicity Factor, y_t	
			Type of Fish	Length cm	Weight g	K x 10^2 g/cm^3	Experimental*	Theoretical**
1	Missouri River at St. Louis CCE	36.0	Trout	5.3	2.8	1.86	1,930	1,890
2		32.0		5.3	2.8	1.86	1,720	1,690
4		27.6		5.3	2.8	1.86	1,500	1,360
5		24.0		5.3	2.8	1.86	1,300	1,230
10		10.0		5.3	2.2	1.48	680	700
13		7.5		5.3	2.2	1.48	540	500
19		4.2		5.3	2.2	1.48	280	290
30		--		--	--	--	--	110
40		--		--	--	--	--	60
60		--		--	--	--	--	30
80		--		--	--	--	--	30
1	Malathion	0.0050		10.0	15.0	1.50	0.373	0.373
2		0.0046		10.0	15.0	1.50	0.306	0.297
4		0.0028		10.0	15.0	1.50	0.186	0.188
5		0.0023		10.0	15.0	1.50	0.153	0.151
7		0.0010		6.0	3.8	1.76	0.057	0.060
15		0.0075		6.0	3.8	1.76	0.043	0.038
30		--		--	--	--	--	0.010
40		--		--	--	--	--	0.005
60		--		--	--	--	--	0.001

*Calculated using Equation 6.

**Calculated using Equation 7 for surface water CCE and Equation 8 for malathion.

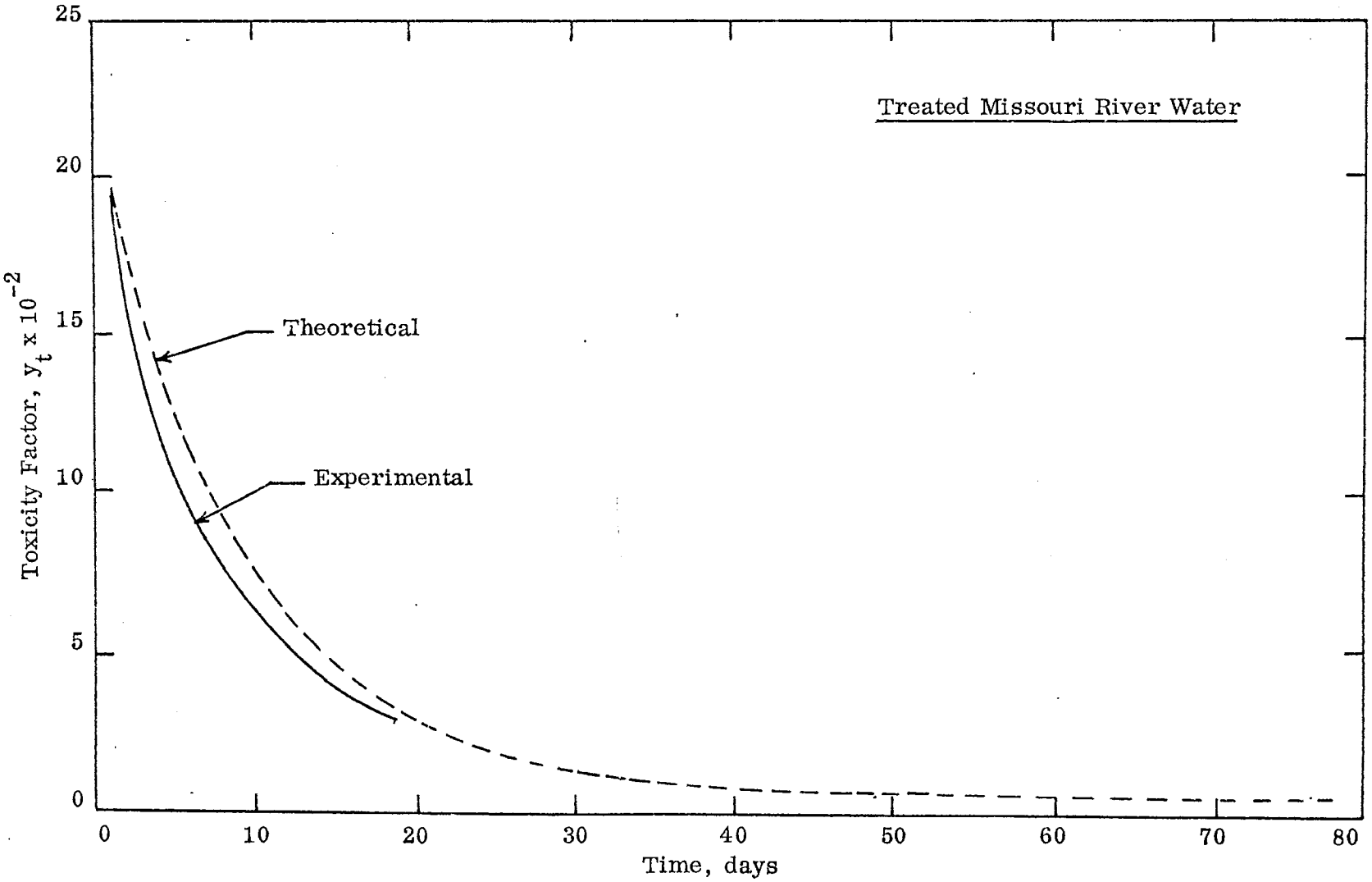

Figure 17

Toxicity Curves for Trout
Exposed to a Surface Water CCE

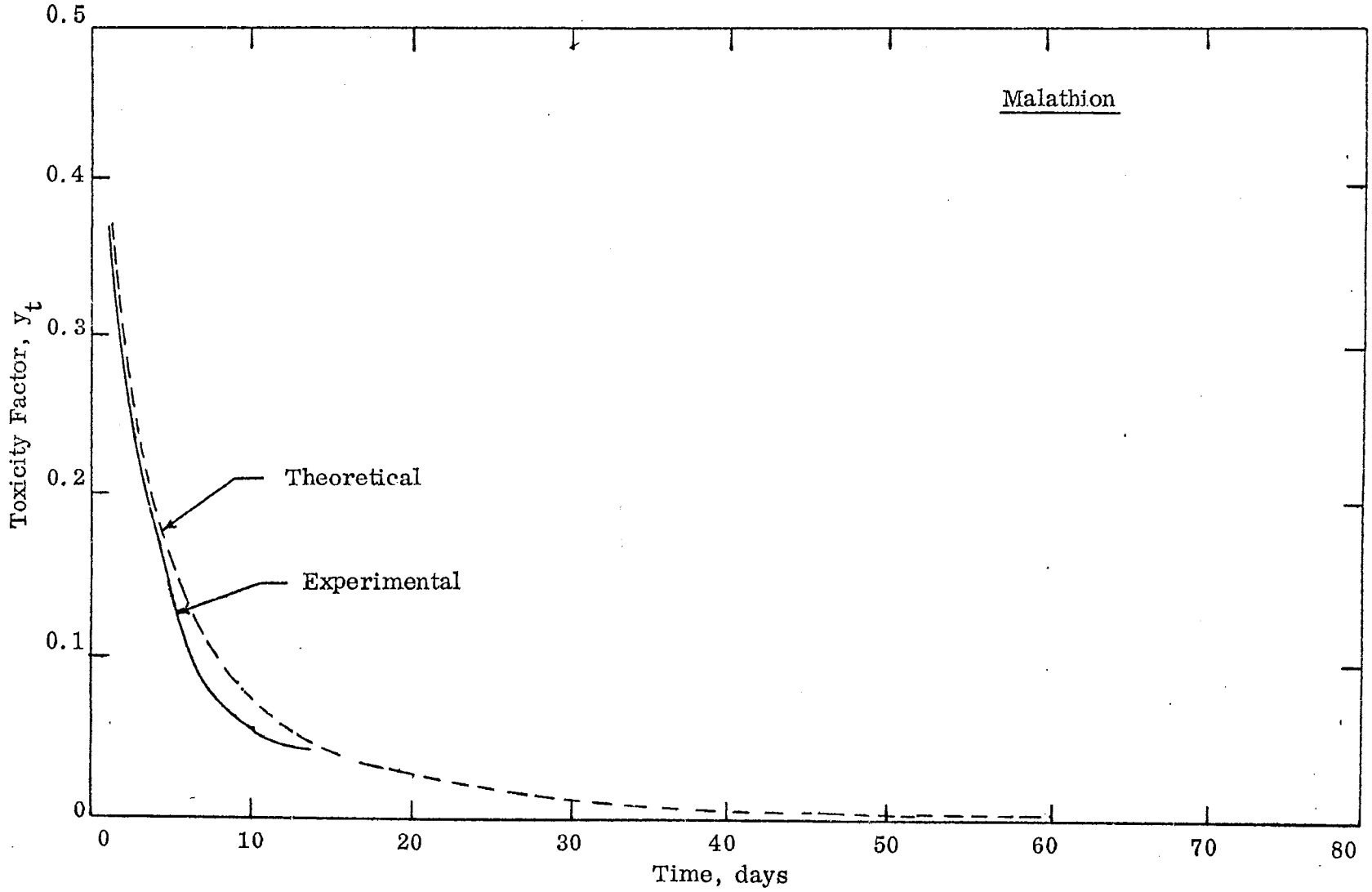

Figure 18

Toxicity Curves for Trout
Exposed to a Pesticide

y_t values (Table 19) for various concentrations and from these values develop, using the procedure outlined in Appendix D, the corresponding toxicity equation:

$$y_t = 4.0 \times 10^2 + (56.0 \times 10^2)\, e^{-0.38t} \qquad \text{(Equation 9)}$$

This equation was used to determine the theoretical toxicity factor values which are presented in Table 19 and in Figure 19. The theoretical curve approximated the experimental reasonably well and could have been used to estimate the long-term concentration level.

Long-term toxic levels were also evaluated on the basis of acute toxicity data for some of the test materials used in this investigation and in other investigations reported in the literature; the values obtained are presented in Tables 20 and 21, respectively, together with the equation developed for each test material and the corresponding test fish. The toxicity factor for the subsurface water organics was defined as $C_t \cdot \left[\frac{GSA}{K}\right]^{1.25}$, while for the surface water CCE, pesticides, and compounds reported in the literature it was defined as C_t/K.

It should be emphasized that the toxicity factor does not constitute a TLm value, but represents a relationship between the TLm concentration and appropriate physical characteristics of the test fish. However, the TLm value at any time can be computed from the corresponding toxicity factor at the same time and Equation 1 or 6, as appropriate.

The procedure developed for the prediction of long-term toxic levels provided a practical method for the estimation of long-term TLm values on the basis of short-term studies and a knowledge of the mode of action of the toxicant.

Table 19

Toxicity Factors for Test Fish Exposed to Acrylonitrile*

Time days	TLm mg/l	Test Fish Characteristics				Toxicity Factor, $y_t \times 10^{-2}$	
		Type of Fish	Length cm	Weight g	K g/cm^3	Experimental**	Theoretical***
1	34	Flathead Minnows	5.7	1.5	0.008	42.5	42.4
2	22		5.7	1.5	0.008	27.5	30.2
3	18		5.7	1.5	0.008	22.5	22.0
4	10		5.7	1.5	0.008	12.5	16.3
5	8.0		5.7	1.5	0.008	10.0	12.2
10	7.0		5.7	1.5	0.008	8.8	5.2
15	5.0		5.7	1.5	0.008	6.3	4.2
20	4.0		5.7	1.5	0.008	5.0	4.0
25	3.5		5.7	1.5	0.008	4.4	4.0
30	2.6		5.7	1.5	0.008	3.3	4.0

*Based on data reported by Henderson, et al. (69).
**Calculated using Equation 6.
***Calculated using Equation 9.

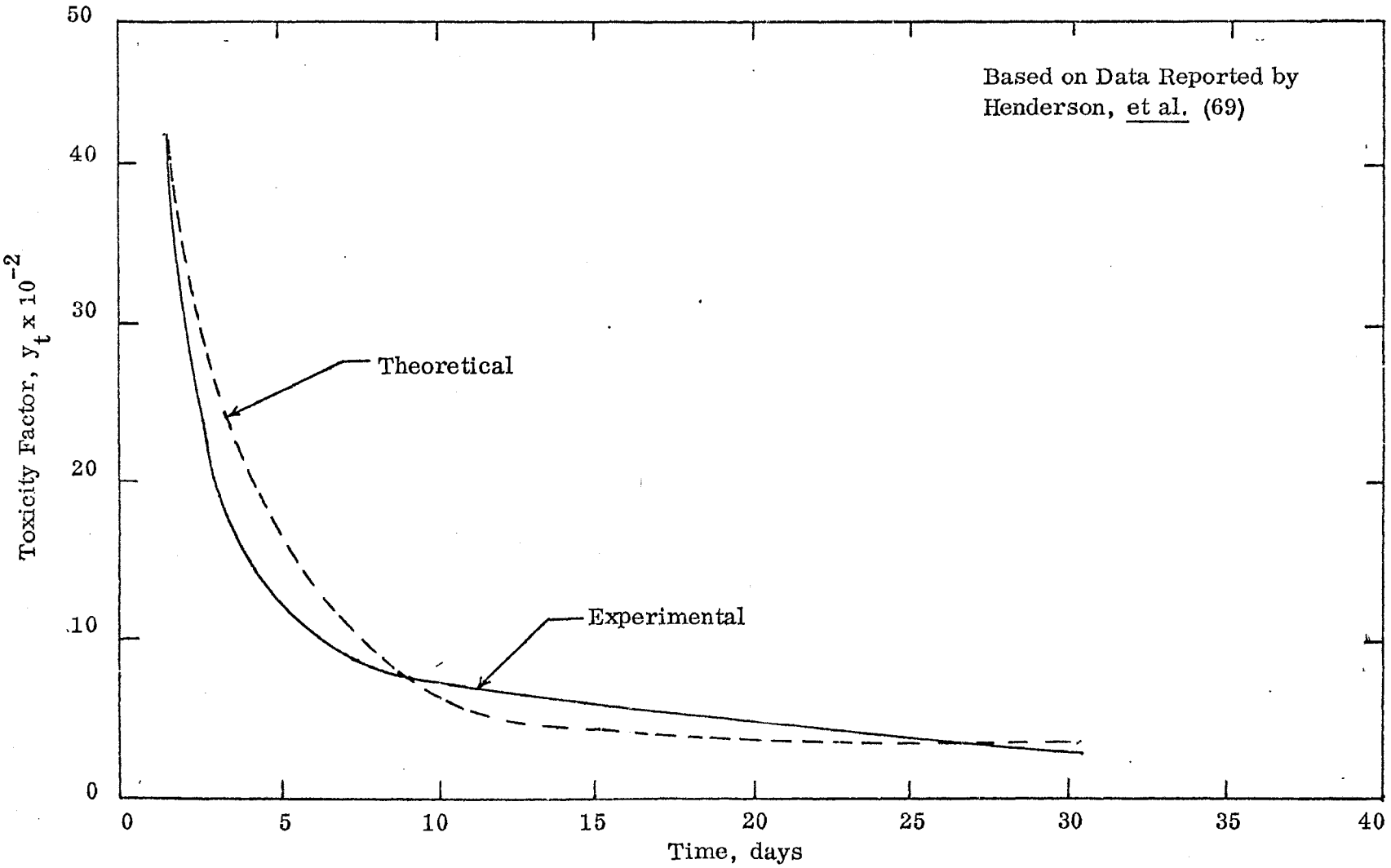

Figure 19

Toxicity Curves for Flathead Minnows
Exposed to Acrylonitrile

Table 20

Theoretical Long-Term TLm Values
For Organic Micropollutants and Pesticides

Test Material	Test Fish	y_t Defined as	Toxicity Equation	Long-Term TLm Reached at Time t
Meramec Spring Run #2 Unit #1	Trout	$C_t \cdot \left[\frac{GSA}{K}\right]^{1.25}$	$y_t = 0.85 \times 10^7 + (2.95 \times 10^7)\, e^{-0.17t}$	33.1 mg/l - 40 days
	Sunfish		$y_t = 1.8 \times 10^7 + (11.6 \times 10^7)\, e^{-0.15t}$	25.4 mg/l - 50 days
Unit #2	Trout		$y_t = 0.10 \times 10^7 + (2.7 \times 10^7)\, e^{-0.11t}$	3.9 mg/l - 70 days
	Sunfish		$y_t = 4.2 \times 10^7 + (9.0 \times 10^7)\, e^{-0.13t}$	47.0 mg/l - 50 days
	Red Shiners		$y_t = 4.0 \times 10^7 + (82.0 \times 10^7)\, e^{-0.11t}$	10.3 mg/l - 50 days
	Golden Shiners		$y_t = 47.5 \times 10^7 + (18.0 \times 10^7)\, e^{-0.32t}$	141.0 mg/l - 20 days
Missouri River at St. Louis CCE	Trout	C_t/K	$y_t = 0.30 \times 10^2 + (20.7 \times 10^2)\, e^{-0.11t}$	0.44 mg/l - 80 days
	Sunfish		$y_t = 11.8 \times 10^2 + (13.2 \times 10^2)\, e^{-0.27t}$	30.1 mg/l - 30 days
	Golden Shiners		$y_t = 5.0 \times 10^2 + (41.0 \times 10^2)\, e^{-0.18t}$	7.5 mg/l - 40 days
Malathion	Trout		$y_t = 0.10 \times 10^{-2} + (46.7 \times 10^{-2})\, e^{-0.23t}$	0.02 μg/l - 60 days
	Red Shiners		$y_t = 1.90 \times 10^{-1} + (24.8 \times 10^{-1})\, e^{-0.15t}$	3.3 μg/l - 50 days
Sevin	Trout		$y_t = 0.20 \times 10^2 + (2.06 \times 10^2)\, e^{-0.44t}$	0.3 mg/l - 20 days
	Red Shiners		$y_t = 1.4 \times 10^2 + (9.1 \times 10^2)\, e^{-0.18t}$	2.0 mg/l - 40 days

Table 21

Theoretical Long-Term TLm Values for Several
Toxic Materials Reported in the Literature

Test Material	Test Fish	y_t Defined as	Toxicity Equation	Long-Term TLm Reached at Time t	Reference
Sevin	Golden Shiners	C_t/K	$y_t = 9.5 \times 10^2 + (74.9 \times 10^2)\ e^{-0.42t}$	8.8 mg/l - 20 days	63
Toluene	Guppies		$y_t = 2.0 \times 10^3 + (2.55 \times 10^3)\ e^{-0.04t}$	28.2 mg/l - 140 days	72
3-Chloroprene	Guppies		$y_t = 2.4 \times 10^3 + (1.88 \times 10^3)\ e^{-0.10t}$	34.0 mg/l - 60 days	72
Adiponitrile	Sunfish		$y_t = 1.15 \times 10^4 + (3.85 \times 10^4)\ e^{-0.30t}$	359.0 mg/l - 30 days	69
Benzonitrile	Flathead Minnows		$y_t = 5.0 \times 10^3 + (32.0 \times 10^3)\ e^{-0.25t}$	40.0 mg/l - 30 days	69
Acryonitrile	Flathead Minnows		$y_t = 4.0 \times 10^2 + (56.0 \times 10^2)\ e^{-0.38t}$	3.2 mg/l - 20 days	69

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Although the development of the method was based on batch-type studies, it should be equally applicable to continuous flow studies, as illustrated by the successful approximation of the long-term findings of Henderson, et al. (69). This is not intended to imply that the mathematical model can be used to predict the exact experimental long-term values, but it does provide an estimation of the range in which the toxicity level should fall.

V. IDENTIFICATION STUDIES

Studies were undertaken to establish the functional groups and character of the components of the complex organic micropollutants. Knowledge of the identity of these materials would assist in the evaluation of their long-term toxic effects, especially with regard to man, and in the development of effective control methods. The use of sensitive sophisticated instrumentation was required in these studies because ordinary characterization techniques revealed very little of the identity of these materials. Gas-liquid chromatography and infrared analysis, individually and in combination, were, therefore, employed; and an effort was made to develop the necessary methodology so that it can be successfully employed in the water and waste water treatment field.

A. GAS CHROMATOGRAPHIC STUDIES

Gas-liquid chromatography can be employed both as an analytical technique for qualitative or quantitative analysis and as a preparative technique for isolation of the components of a complex material and requires minute quantities of sample (as low as 50 μg/l) when a hydrogen flame detector is used.

A Varian Aerograph dual column, dual hydrogen flame gas chromatograph* (Figure 20) equipped with a Leeds & Northrup recorder** was utilized to characterize and to separate the trace organics. Single column operation was used in all analyses, and the procedure employed consisted of injecting an

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*Model 1520-1B, a product of Varian Aerograph, Walnut Creek, Calif.
**Speedomax W recorder, a product of the Leeds & Northrup Company, Philadelphia, Pa.

Figure 20

Varian Aerograph Model 1520-1B Gas Chromatograph
And Leeds & Northrup Speedomax W Recorder

appropriate size sample (4 or 50 μl) into the injector port where it was vaporized and mixed with nitrogen carrier gas; the mixture of vaporized sample and carrier gas then passed into a stainless steel column (1/8 or 1/4 inch in diameter) containing a high temperature liquid solvent (phase) on a solid support; part of the vaporized sample was condensed in the column and part was carried through the column to a hydrogen flame detector. This detector consisted of a hydrogen flame burning in a closed chamber under forced aeration; as the components were eluted from the column and passed through the flame, combustion of the organic material resulted in the release of negative ions causing a change in the electrical potential between two electrodes located above the flame; the change in potential was then indicated on a strip chart recorder.

The three operating parameters which were varied in the gas-liquid chromatographic studies were the column oven temperature, the rate of increase of the oven temperature, and the liquid phase or support in the column. The oven temperature was varied from ambient (26^{o}C) to near the maximum permissible temperature for the liquid phase in a column in order to determine the optimum operating temperature range. It was found, however, that the organic micropollutants vaporized over a very wide temperature range (75 to 275^{o}C for Meramec Spring Run #2 Unit #1 CCE) which made isothermal operation inapplicable because of the time required for complete separation. The oven temperature was, therefore, increased at a linear rate from an initial temperature of 75^{o}C to a final temperature which was 20^{o}C less than the maximum permissible temperature for the column being employed (Table 22).

Table 22

Liquid Phases and Test Conditions
Employed in the Gas Chromatographic Studies

Liquid Phase*				Maximum Permissible Temperature °C	Test Conditions Evaluated °C
Type	Designation	Polarity	Per Cent Loading**		
Carbowax 20 M	--	Polar	10	250	LTP‡ from 26 to 230 Iso.‡ @ 26, 100, 230
Diethylene Glycol Succinate (DEGS)	DEGS	Polar	20	190	LTP from 26 to 170 Iso. @ 26, 100, 170
FFAP	FFAP	Polar, Specific	10	275	LTP from 26 to 255 Iso. @ 26, 100, 255
Hallcomid M-18-0L	--	Intermediate	20	150	LTP from 26 to 130 Iso. @ 26, 100, 130
Porapak Q	--	--	‡‡	240	LTP from 26 to 220 Iso. @ 26, 100, 220
Silicone (Fluoro) QF-1 (FS 1265)	QF-1	Intermediate	5	250	LTP from 26 to 230 Iso. @ 26, 100, 230
Silicone GE (Versilube F-50)	Versilube F-50	Intermediate	10	300	LTP from 26 to 280 Iso. @ 26, 100, 280
Silicone Gum Rubber SE-30 (methyl)	SE-30	Non Polar	10	375	LTP from 26 to 355 Iso. @ 26, 100, 200, 355

*All procured from Varian Aerograph.
**Solid support was 60/80 mesh Chromosorb W.
‡LTP: Linear Temperature Programming; Iso.: Isothermal.
‡‡Porapak served both as liquid and solid support.

A temperature higher than this was not used since it would have reduced the life of the column and caused excessive column bleed. Linear temperature programming of the column oven temperature was possible with this instrument at rates ranging from 2 to 40°C per minute. The oven temperature was maintained at 75°C for 2 minutes following the injection of the sample to allow elution of the solvent and was then linearly programmed at a rate selected to effect good separation to the upper limit, where it was held isothermally until all the sample had been eluted from the column. The injector and detector ovens were maintained 20°C higher than the maximum column oven temperature to insure that the sample would be volatilized before it reached the column and would remain in the vapor phase while going through the detector.

The third operating parameter which was varied was the liquid phase in the column. This phase determined to a large degree the extent of the separation possible. Eight different liquid phases* were evaluated as column packing materials (Table 22). Of these, only three, Versilube F-50, QF-1, and FFAP, were found to separate the chloroform and acetone soluble organics; QF-1 and FFAP columns gave good separations and were used in the studies. None of the eight columns tested was capable of separating the alcohol soluble material. Assistance was sought from the Varian Aerograph Applications Laboratory** in finding a liquid phase and corresponding operating conditions

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*All these materials were procured from Varian Aerograph, Walnut Creek, Calif.

**Varian Aerograph, 2700 Mitchell Avenue, Walnut Creek, Calif.

capable of effecting the separation of the CAE organics. Unfortunately, they also were unable to separate this material and could only suggest that in their opinion the alcohol soluble material was not amenable to separation by gas-liquid chromatographic techniques (74). Difficulty was also encountered in the separation of the benzene soluble organics.

Although the gas chromatograph was not used continuously, the injector and detector ovens, as well as the electronic circuits of the temperature programmer and signal attenuator, were allowed to remain on. The instrument could be, therefore, operated at any time since electrical warm-up was not necessary. The hydrogen and air flow rates were set at 40 and 200 ml per minute, respectively; these rates gave good flame stability and low background noise. The nitrogen (carrier gas) flow rate was set at 25 ml per minute. High purity hydrogen* and prepurified nitrogen* were supplied from large size gas cylinders. Air was supplied by two diaphragm-type aquarium pumps.

Stock solutions containing 20 mg/ml of organics were prepared using acetone as the solvent for the CCE and CAcE materials and the group breakdown fractions. A 4 μl sample was injected into the 1/8 inch columns; and the attenuation was adjusted to keep the major portion of the large peaks on the graph without, however, masking out the weaker peaks. After the attenuation had been properly adjusted, two additional samples were injected to check

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*Procured from two suppliers: The Matheson Company, Inc., Jolliet, Ill., and the Central Stores, University of Missouri - Columbia, Columbia, Mo.

reproducibility. Following each analysis, 4 μl solvent was injected into the column and the column heated to its upper operating limit and maintained at that temperature for 5 minutes to remove any material which might have not been eluted from it.

The complexity of the chloroform and acetone soluble trace organics is illustrated by the representative chromatograms shown in Figures 21 through 24. Theoretically, each peak on the chromatogram should represent one compound; however, this may not actually be true. If several compounds were present which vaporized at the same, or very nearly the same, temperature, more than one of these substances could have been eluted from the column at the same time, and, therefore, detected as the same peak. Although the selective liquid support in the column was designed to prevent this from happening, it could not be expected to be completely effective in all cases. Analysis of the samples on a second liquid phase was attempted to alleviate grouping, but this could have also occurred on the second column. In an attempt to reduce the complexity of the sample, solubility fractions obtained by group breakdown (see Figure 2, p. 46) were also analyzed and the chromatograms obtained are presented in Figures 21 through 24 with the parent CCE.

Although some of the fractions* could not be separated with the columns utilized and did not give a peak, the neutrals in every case could be separated and appeared to be complex materials with many peaks. The character of the

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*Notably amphoterics and bases and other fractions depending on the particular extract investigated.

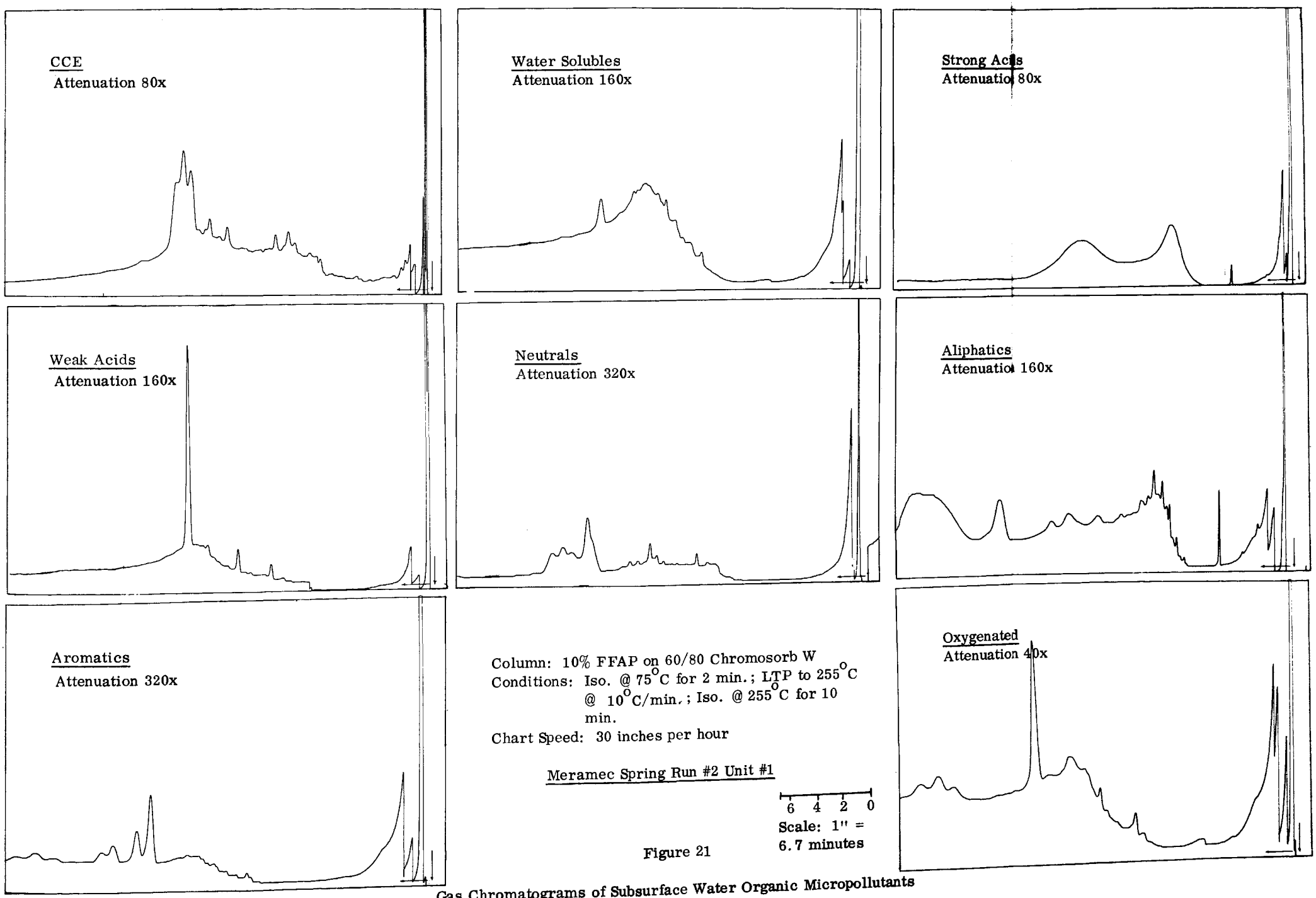
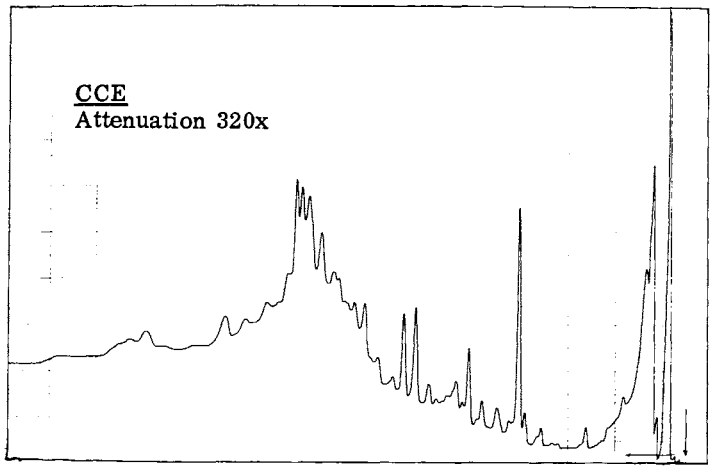
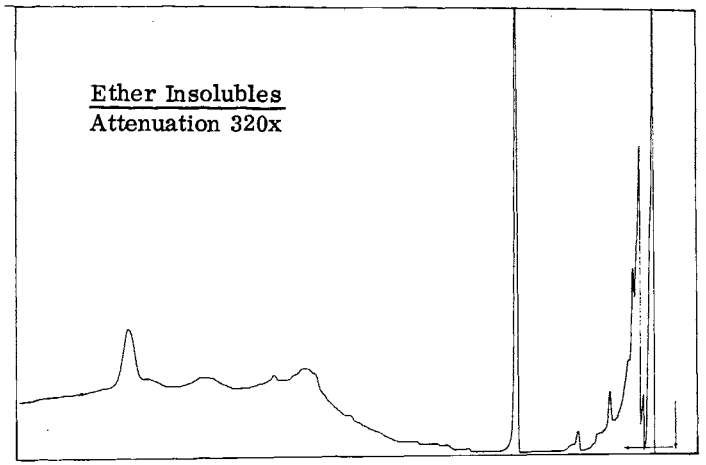
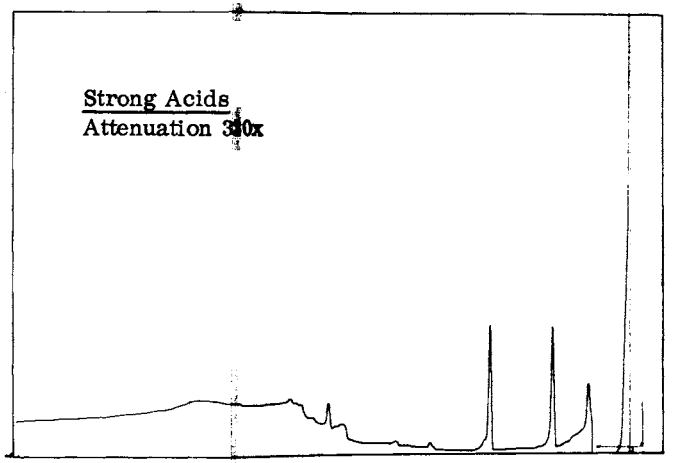


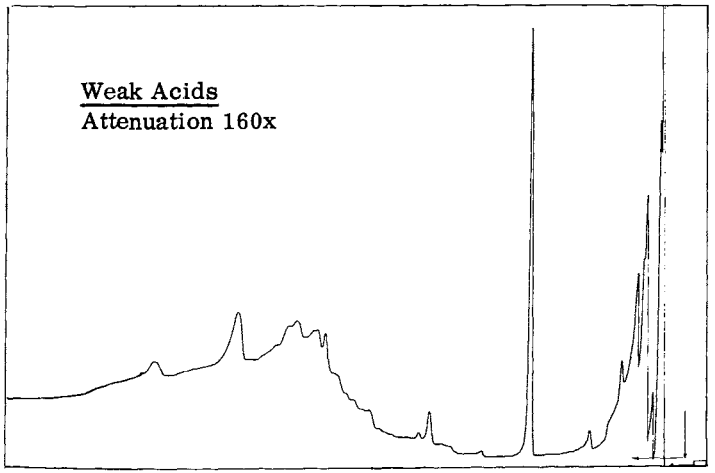
Figure 21

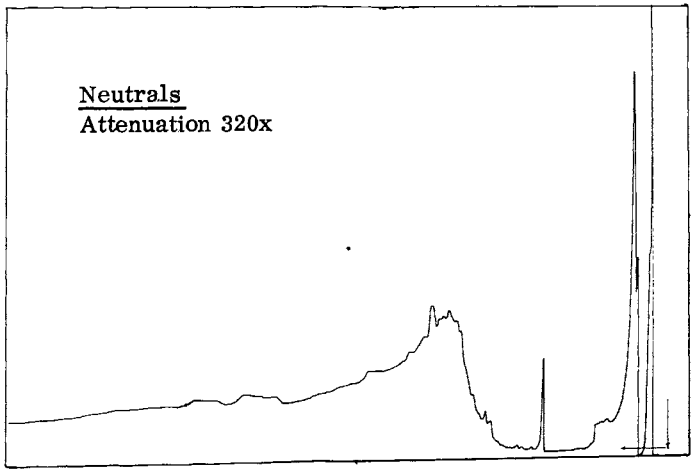
Gas Chromatograms of Subsurface Water Organic Micropollutants Obtained with a 10% FFAP Column

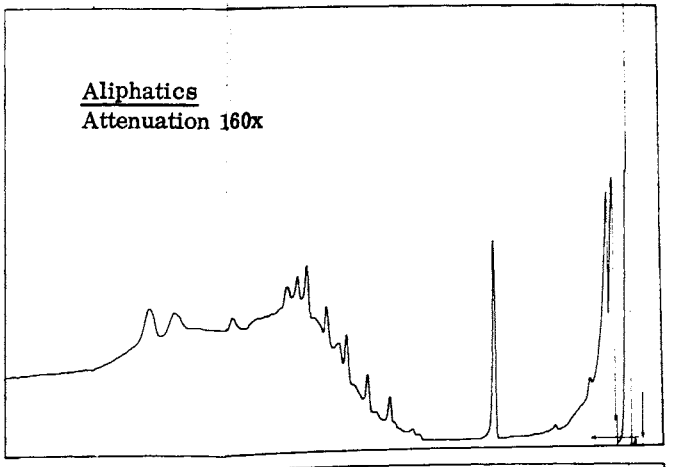


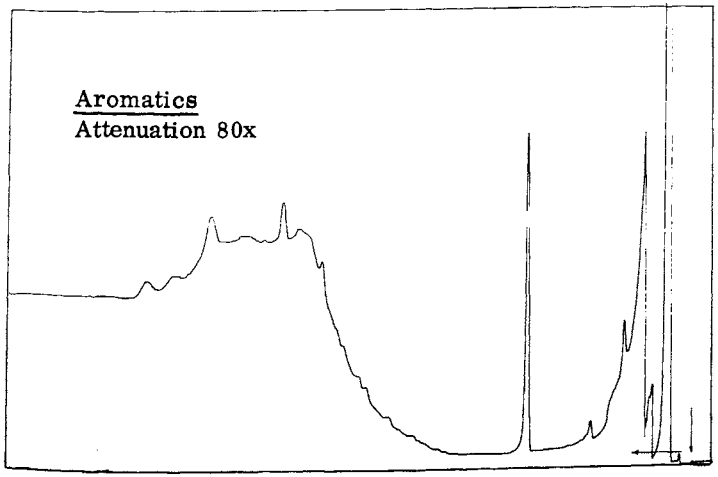












Column: 10% FFAP on 60/80 Chromosorb W
Conditions: Iso. @ 75°C for 2 min.; LTP to 255°C @ 10°C/min.; Iso. @ 255°C for 10 min.
Chart Speed: 30 inches per hour
Note: All fractions obtained by group breakdown of CCE

UMR Well

6 4 2 0
Scale: 1" = 6.7 minutes

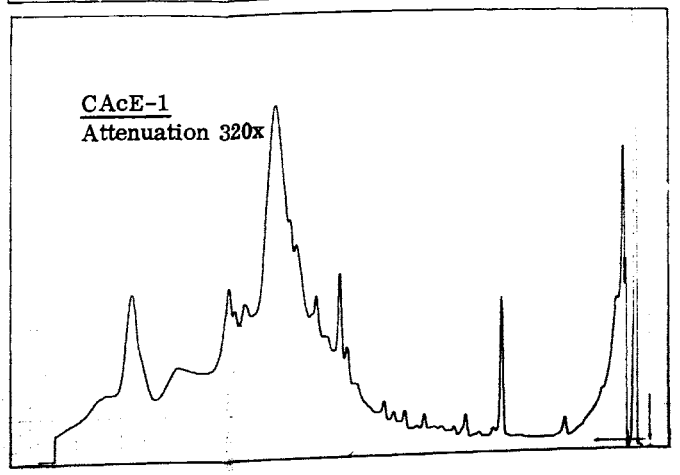


Figure 21 (Continued)

Gas Chromatograms of Subsurface Water Organic Micropollutants Obtained with a 10% FFAP Column

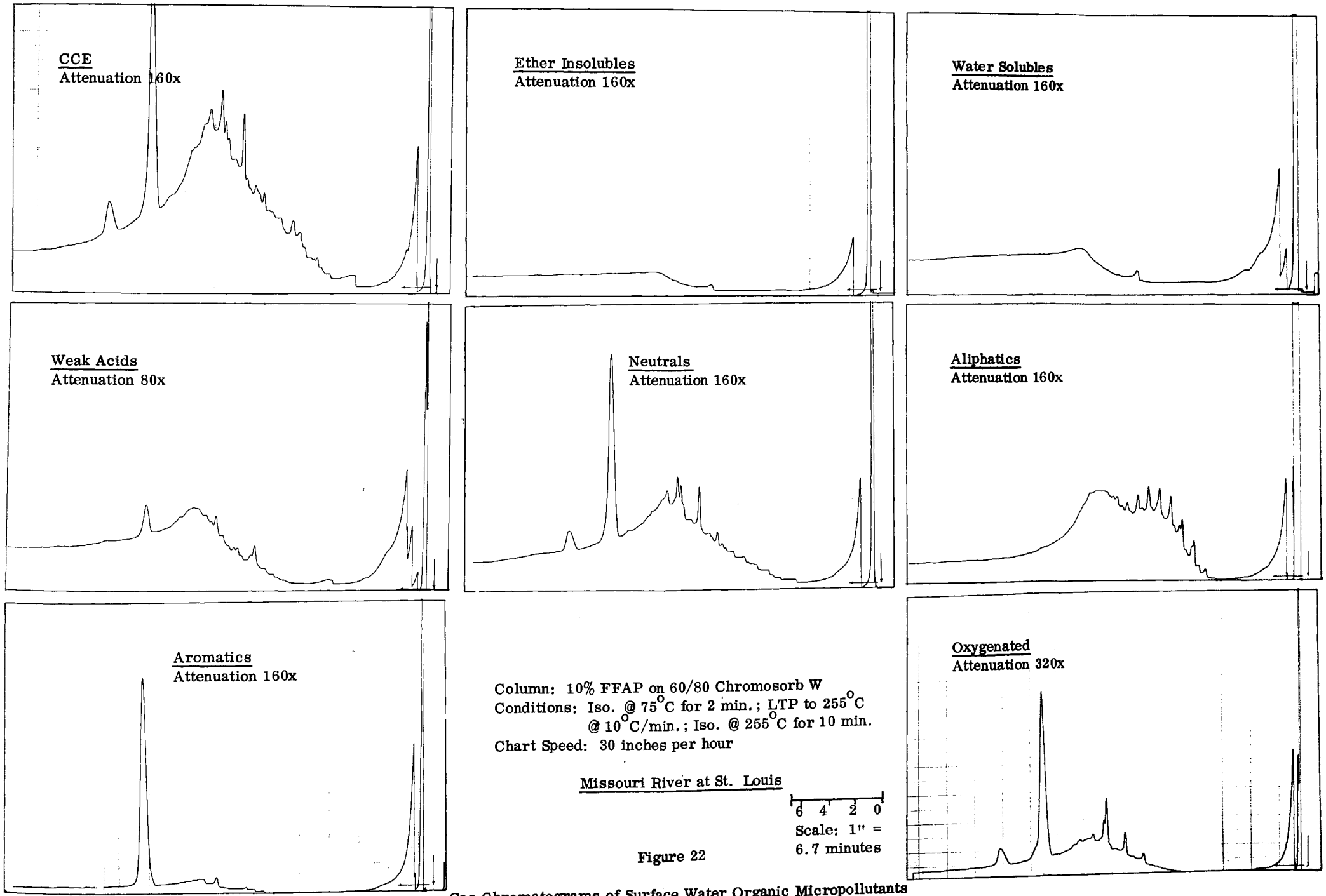
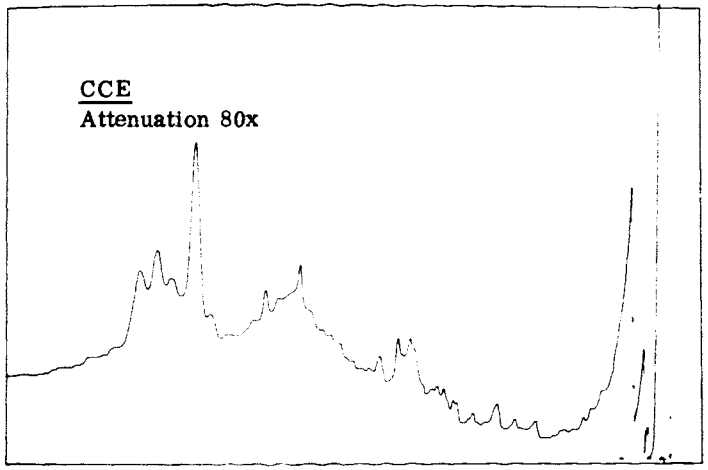
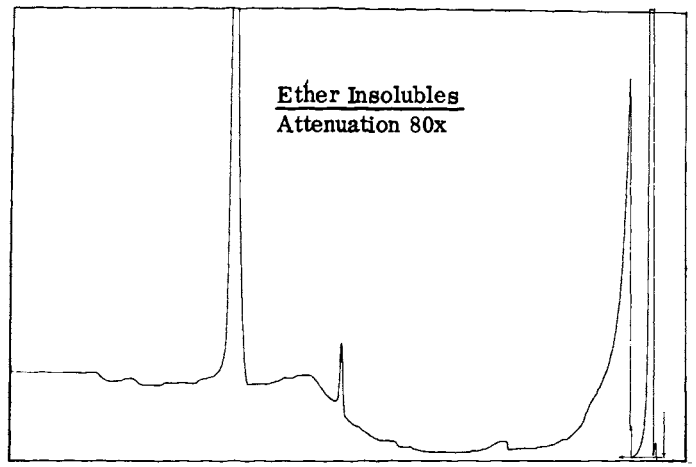
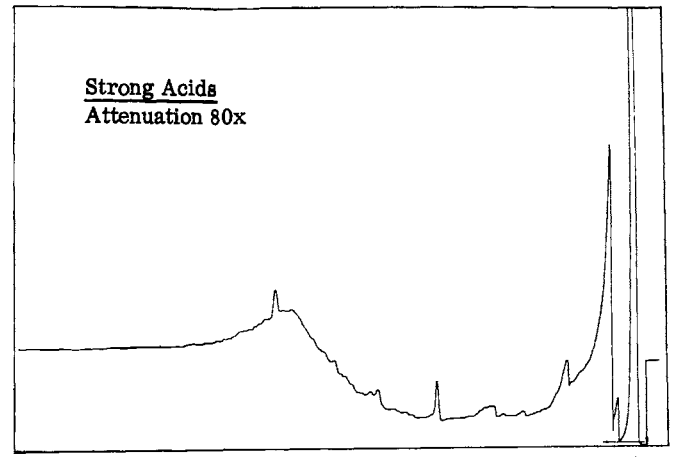


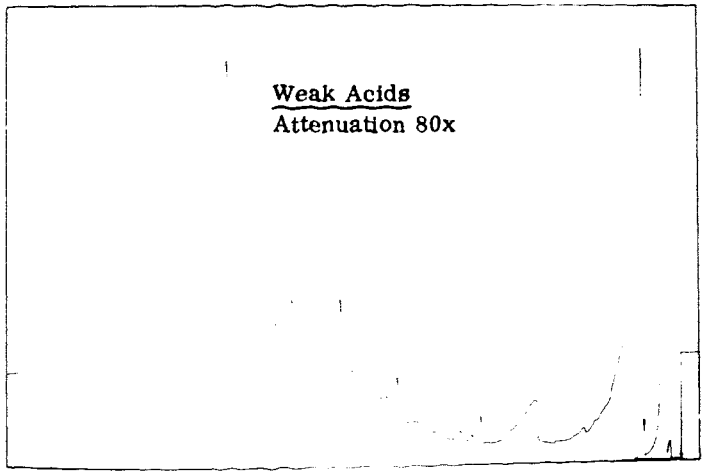
Figure 22

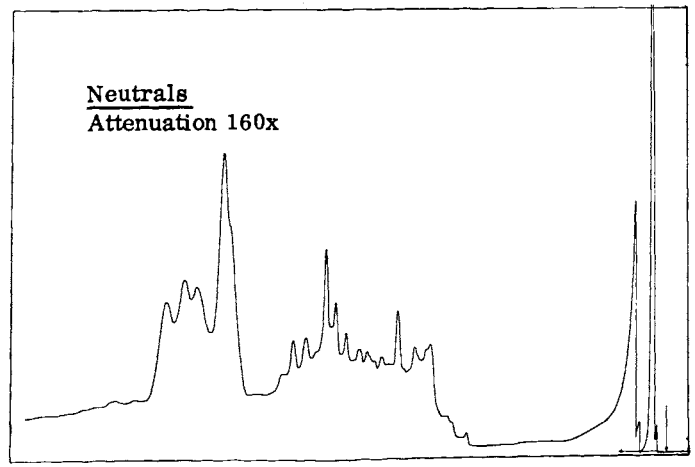
Gas Chromatograms of Surface Water Organic Micropollutants
Obtained with a 10% FFAP Column

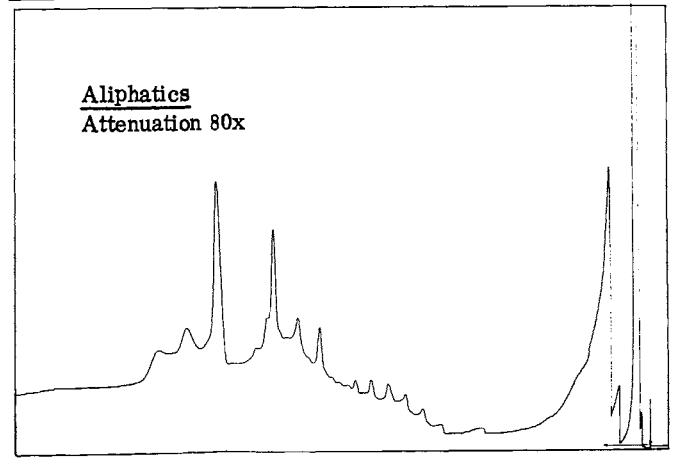


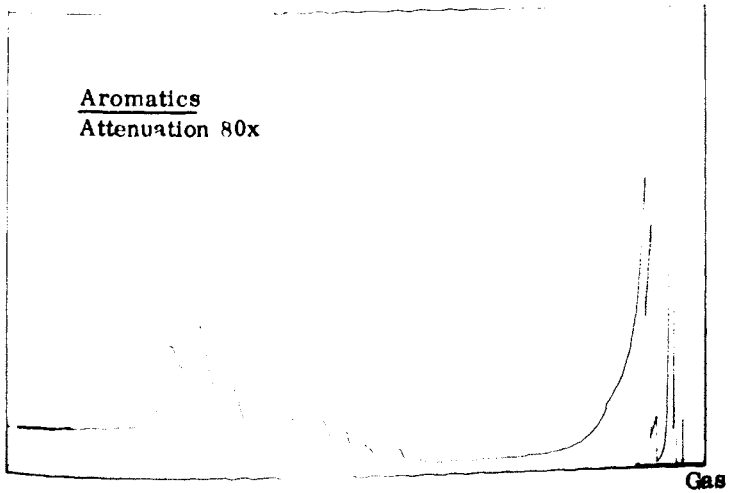






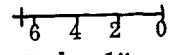






Column: 5% QF-1 on 60/80 Chromosorb W
Conditions: Iso. @ 75°C for 2 min.; LTP to 230°C @ 10°C per min; Iso. @ 230°C for 8 min.
Chart Speed: 30 inches per hour

Meramec Spring Run #2 Unit #1



Scale: 1" = 6.7 minutes

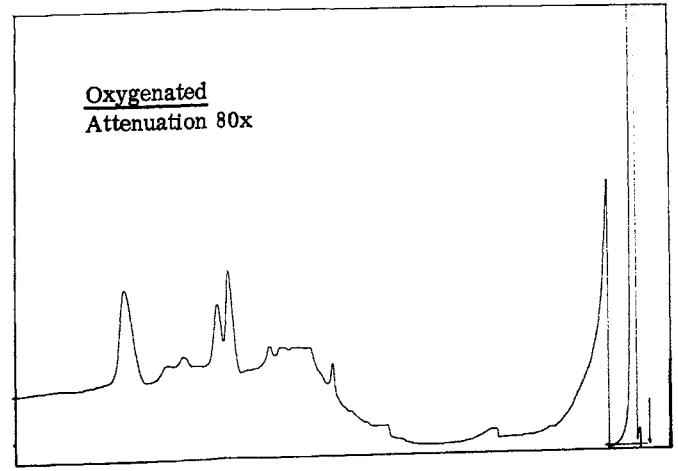
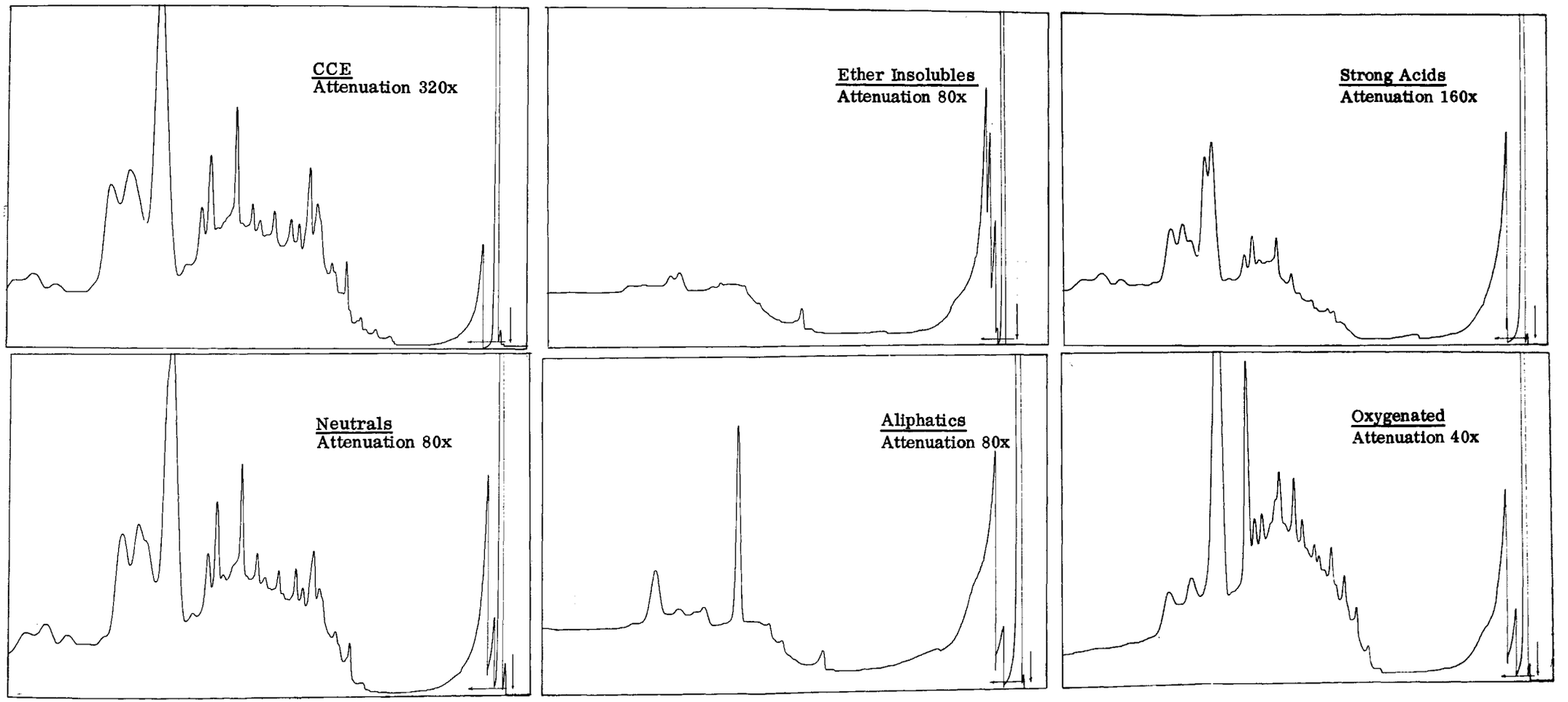


Figure 23

Gas Chromatograms of Subsurface Water Organic Micropollutants Obtained with a 5% QF-1 Column



Column: 5% QF-1 on 60/80 Chromosorb W
Conditions: Iso. @ 75°C for 2 min.; LTP to 230°C @ 10°C/min.; Iso. @ 230°C for 8 min.
Chart Speed: 30 inches per hour

UMR Well

6 4 2 0
Scale: 1" = 6.7 minutes

Figure 23 (Continued)

Gas Chromatograms of Subsurface Water Organic Micropollutants
Obtained with a 5% QF-1 Column

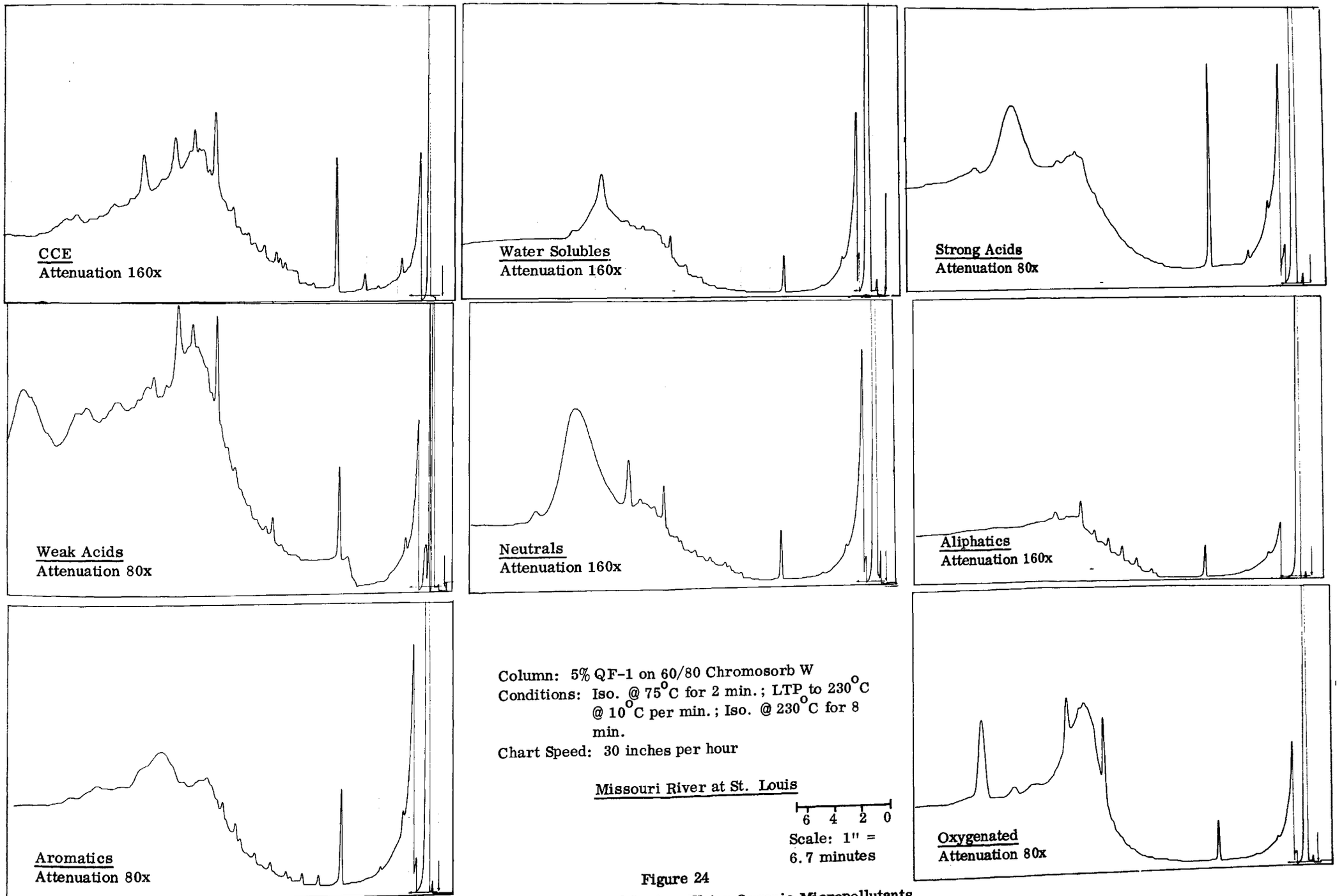


Figure 24

Gas Chromatograms of Surface Water Organic Micropollutants Obtained with a 5% QF-1 Column

neutrals was further investigated by subjecting fractions obtained by column chromatographic separation of the neutrals (Figure 2) to analysis. Most of these fractions could be separated by the gas chromatograph with the aliphatics showing the largest number of peaks and the corresponding chromatograms are also included in Figures 21 through 24.

To facilitate evaluation of the chromatograms, relative retention times were calculated for all peaks which were at least twice the size of the maximum noise and are presented in Tables 23 and 24. The leading edge of the solvent was used as a reference point. It should be pointed out that even though temperature programming was employed to speed up the analysis, comparison of the retention time of the various peaks in different samples was possible because of the ability of the programmer to reproduce programming rates. The values given in Tables 23 and 24 were utilized to compare the total number of peaks in the parent CCE and its fractions, and should not be construed to represent all the compounds present in the organic micropollutants. If the column were performing at peak efficiency, the number of peaks in the parent CCE chromatogram should have been equal to the number of peaks of all the fractions; the same relationship should have applied to the neutrals and their fractions. However, this was not found to be true and a considerable number of peaks with retention time different from the peaks in the parent compound was found in the fractions. It should be emphasized that group breakdown provided less complex samples for analysis; however, at the same time, it concentrated substances which may have shown up as minor peaks in the parent compound resulting in these substances being represented as major peaks in the chromatogram

Table 23

Relative Retention Time of Chromatogram Peaks Resulting From
Gas-Liquid Chromatographic Analysis of Organic Micropollutants with a 10% FFAP Column

Test Material	Attenuation	Relative Retention Time, Minutes																								
Meramec Spring Run #2 Unit #1																										
CCE	320x	2.1	2.4				11.7		13.2		15.0	16.0		16.9				20.1	21.3	22.6	23.1					
Ether Insolubles	160x		2.5	3.8	5.5	10.0		12.2																		
Water Solubles	80x						11.8			14.4		15.7					19.0									
Weak Acids	160x																19.0		21.3		23.2		25.7			
Neutrals	320x							12.2		14.6	15.0	15.5	16.4	17.0				19.9	21.6	22.4						
Aliphatics	160x			3.7	6.0	9.2				14.3	15.3		16.3	17.3	18.3	18.7	19.2		20.6	22.2		24.3	25.5		29.4	
Aromatics	80x						11.9										19.0	19.9	21.5	22.3				26.8		
Oxygenated	40x				7.0			12.5		14.5			16.6				19.0						25.1	26.2	27.5	
UMR Well Unit #1																										
CCE	320x		1.5	2.4	4.0	6.6	6.9	7.7	8.4	9.0	9.5	10.7	11.2	11.7	12.2	12.8	13.4	13.8	14.7	15.2	15.7	16.0	16.3	17.5		19.4	22.6
Ether Insolubles	320x		1.3	2.4	3.8	6.5															15.8					23.5
Strong Acids	320x			2.4			6.8												14.3							
Weak Acids	160x			2.7	4.0	6.5						10.7								15.2			16.3		18.8		22.3
Neutrals	320x				3.5			7.9		9.0	9.5								14.3					17.4		
Aliphatics	160x			2.4	3.9		6.7						11.4		12.4		13.3		14.3	15.2	15.7		16.2		18.2	21.5	22.5
Aromatics	80x			2.8	4.3		6.9																16.5	17.1		20.2	22.9
CAcE-1	320x	1.0	1.4		3.7	6.5			8.2			10.1	11.0	11.5	12.0		13.5	13.8		15.0	15.9			16.9	18.3	19.1	23.4
Missouri River at St. Louis																										
CCE	160x	2.3		8.3			9.5	9.9			11.9	12.5	13.3		14.5	14.7	15.5		19.5		22.3					
Ether Insolubles	160x										11.9															
Water Solubles	160x										12.0															
Weak Acids	80x											13.0		14.2	14.4		15.7			20.3						
Neutrals	160x									11.3	11.6	12.8		14.2	14.5	15.1		19.0			21.9					
Aliphatics	160x		7.3	8.1	8.9	9.2		9.7	10.5	11.3	12.1	12.8	13.5	13.8					19.5							
Aromatics	160x														14.6											
Oxygenated	320x										11.7	13.0		14.3	14.6		15.3	19.1			22.2					

Table 24

Relative Retention Time of Chromatogram Peaks Resulting From
Gas-Liquid Chromatographic Analysis of Organic Micropollutants with a 5% QF-1 Column

Test Material	Attenuation	Relative Retention Time, Minutes																							
Meramec Spring Run #2 Unit #1																									
CCE	80x	3.7	5.7	6.6	7.4	8.4		9.2	9.7	9.9	11.1	11.6		12.4				15.6			17.1	20.1	21.1	21.7	22.6	
Ether Insolubles	80x				7.1												14.5					19.2				
Strong Acids	80x	3.6		6.9					9.5				12.1							16.6						
Weak Acids	80x	4.0	6.0			8.3	8.7	9.3					11.9				14.3		16.3		17.1	20.0				
Neutrals	160x						8.8			10.4	11.2		11.9	12.6	13.3	13.6	14.2	14.7	15.2	16.1	16.6	19.6	20.8	21.3	22.2	
Aliphatics	80x									10.2	11.0	11.7		12.5	13.3			14.8	15.7				21.0		22.3	
Aromatics	80x												11.9				14.2				16.8					
Oxygenated	80x			6.8													14.2				17.0	20.4		21.6		23.6
UMR Well Unit #1																									
CCE	320x	7.7	8.5	9.4	10.2	11.0	11.5	12.1	12.5		13.5	14.3	14.7	15.7		17.2	17.7			20.0		21.7	22.9		26.0	27.2	
Ether Insolubles	80x								12.6											20.0	20.5						
Strong Acids	160x											14.5		15.6		17.3		19.0				21.2	22.0	25.2	26.3	
Neutrals	80x			9.3	10.1	11.0	11.4	12.0	12.4		13.4	14.2	14.6	15.5	16.9		17.4		19.5			21.4	22.3	25.5	26.7	
Aliphatics	80x				10.2	11.0	11.7		12.4	13.2	13.5	14.2	14.7	15.6	16.6	17.0	17.5	19.1				21.0	22.4				
Oxygenated	40x								12.3								17.5		19.5			24.9	22.3				
Missouri River at St. Louis																										
CCE	160x	2.7			7.0				10.3	10.7	10.9	11.7		12.7		13.7	14.7	15.2	16.2	17.5	19.5		23.8			
Water Solubles	160x		3.0		7.0											13.8	14.9		16.8		19.5					
Strong Acids	80x		3.0	4.5	7.1														16.4	17.6		21.1	23.9			
Weak Acids	80x	2.7			7.1							11.6	12.0			14.1		15.3	16.9	17.8	19.5	21.9	23.9	28.2		
Neutrals	160x	2.8				7.4						11.3		12.4	13.4			15.6		17.3		21.7	24.3			
Aliphatics	160x		3.1			7.5						11.3	12.2		13.4		14.4	15.3	16.3		18.1					
Aromatics	80x	2.4				7.5	8.0	9.1	10.2			11.3			13.3	13.6	14.4	15.5			18.4					
Oxygenated	80x		3.0		7.2													15.3	16.6	17.8		21.8	24.1			

of the solubility fraction. Although some of the components present in the solubility fraction may have been masked in the chromatogram of the complex organic, it is possible that some of the original materials were changed or modified through solvent-sample interactions during the group breakdown process. Similar interactions and changes would have taken place, however, regardless of the separation technique utilized.

B. INFRARED SPECTROSCOPIC STUDIES

Gas-liquid chromatography is a powerful identification tool under proper conditions. However, when dealing with very complex compounds or mixtures, other identification techniques must be employed to furnish more information about the unknown. Determination of functional groups with infrared spectroscopy can be employed to formulate a general model of the compound being investigated and can aid in gas chromatographic identification.

A Beckman double beam, linear wavenumber grating infrared spectrophotometer* (Figure 25) was employed in these studies. The instrument could be operated over a spectral range of 4000 to 300 cm^{-1} wavenumber (2.5 to 33.3 microns wavelength) at two scanning speeds, a slow speed of 42 minutes and a fast speed of 14 minutes for the entire range. The sensitivity of the instrument could be controlled by adjusting the gain setting or the width of the slits which regulated the amount of light passing through the system. Under normal operating conditions, the slits were opened at a set programmed rate; however,

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*Model IR10, a product of Beckman Instruments, Inc., Fullerton, Calif.

Figure 25

Beckman IR10 Infrared Spectrophotometer

a manual slit override was available. When an analysis was performed, infrared light of different wavelengths was passed through the sample which absorbed some of the light energy at specific wavelengths depending on the number and types of chemical bonds present. Double beam operation enabled differential analysis of a sample in which the absorption due to the cell or solvent was counterbalanced by a reference sample.

The trace organics were analyzed either in solid form using potassium bromide pellets or in solution with a Beckman demountable liquid cell.* Both sodium chloride** and potassium bromide*** were used as window materials in the demountable cells; a 0.1 mm spacer was employed between the windows providing a 0.1 mm pathlength. Small (8 mm diameter) potassium bromide pellets were formed by mixing 0.6 to 1.0 mg of the sample with 60 to 80 mg dried potassium bromide powder, grinding in a mortar and pestle, and pressing the mixture into a pellet using a Wilks Mini-Press.† Approximately 0.1 ml of a 10 per cent solution of the organics was used with the liquid cell. The choice of solvents for the liquid samples was limited since the organics were soluble only in certain solvents and an acceptable solvent should not have interfered with the adsorption of the sample or damaged the cells. Chloroform was found

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*Model F-05, a product of Beckman Instruments, Inc., Fullerton, Calif.

**Model F-05 F-N (range 4000 to 625 cm^{-1}), a product of Beckman Instruments, Inc., Fullerton, Calif.

***Model F-05 F-K (range 4000 to 250 cm^{-1}), a product of Beckman Instruments, Inc., Fullerton, Calif.

†Model MP-2, a product of the Wilks Scientific Corporation, Norwalk, Conn.

very suitable for the CCE, but an acceptable solvent was not found for the CAE, CBE, and CAcE; consequently, these organics were analyzed in solid form.

When the liquid cells were employed, the gain was set to an appropriate level to give good pen movement and the slit override was not used; however, when potassium bromide pellets were employed, the slit override was used to increase the slit width in order to provide enough energy through the smaller size cell window. The slow scanning speed (42 minutes) was used with all samples to give the best possible resolution.

Infrared spectra of several organic micropollutants and group breakdown fractions are presented in Figures 26 and 27. These materials were selected to represent the various trace organics of interest in this study. Because of the similarity of the absorption bands, spectra for all the organics are not presented. The wavenumber, intensity, and shape at peak maximum of the absorption peaks in the spectra shown in Figures 26 and 27 are tabulated in Table 25. Wavenumbers have been determined with the vernier on the instrument to enable more accurate readings. In order to describe consistently the absorption peaks, the intensity and shape were defined using the terminology* suggested by Afremow and Vandeberg (75).

The spectra shown in Figures 26 and 27 and described in Table 25 represent the functional groups of all the compounds present in the complex

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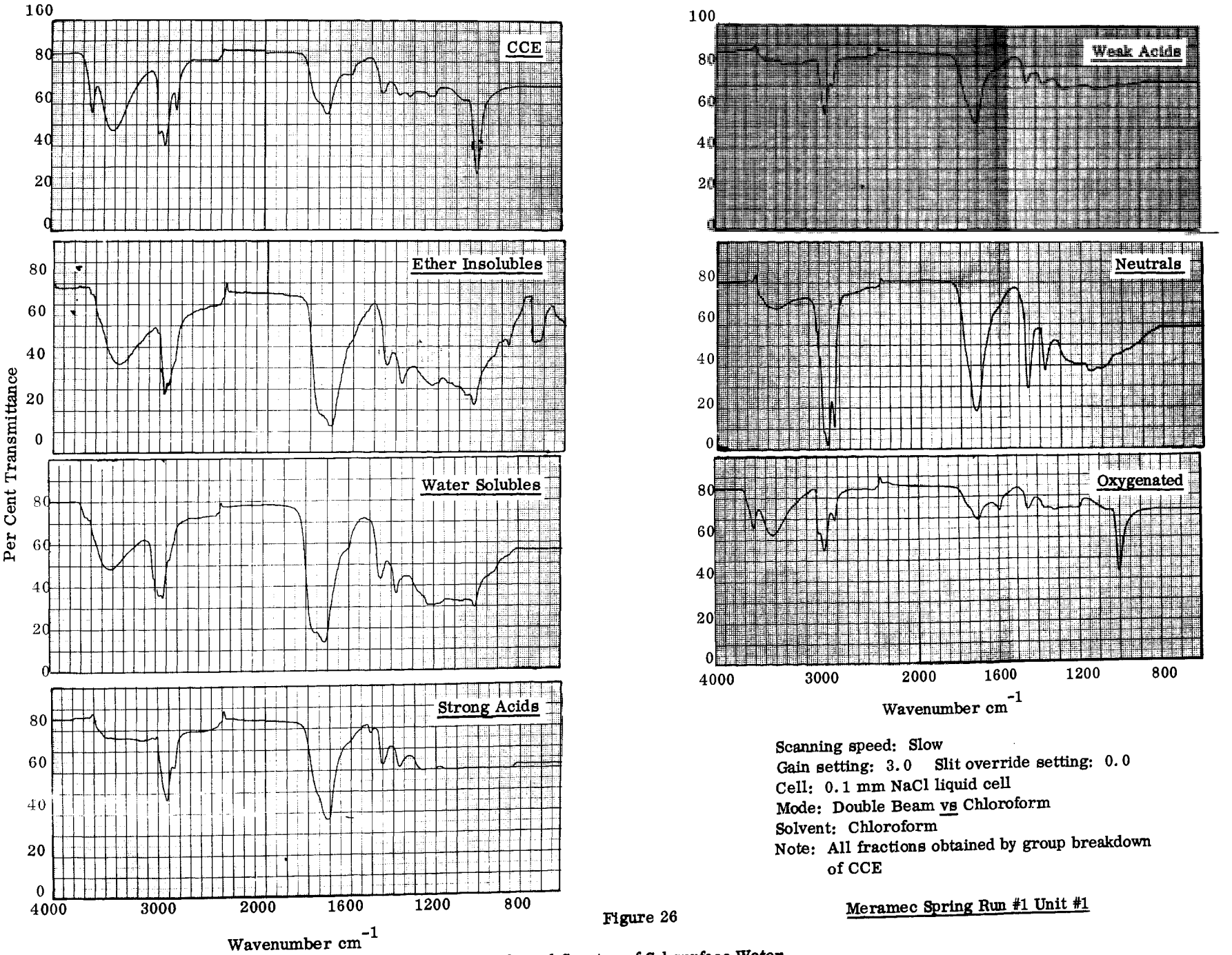
*Intensity was defined as: strong (>35%), medium (10-35%), weak (2-10%), very weak (<2%). Shape at peak maximum defined as: sharp (pen width), medium (pen width to 10 cm^{-1}), broad (10-20 cm^{-1}), very broad (>20 cm^{-1}), shoulder band.

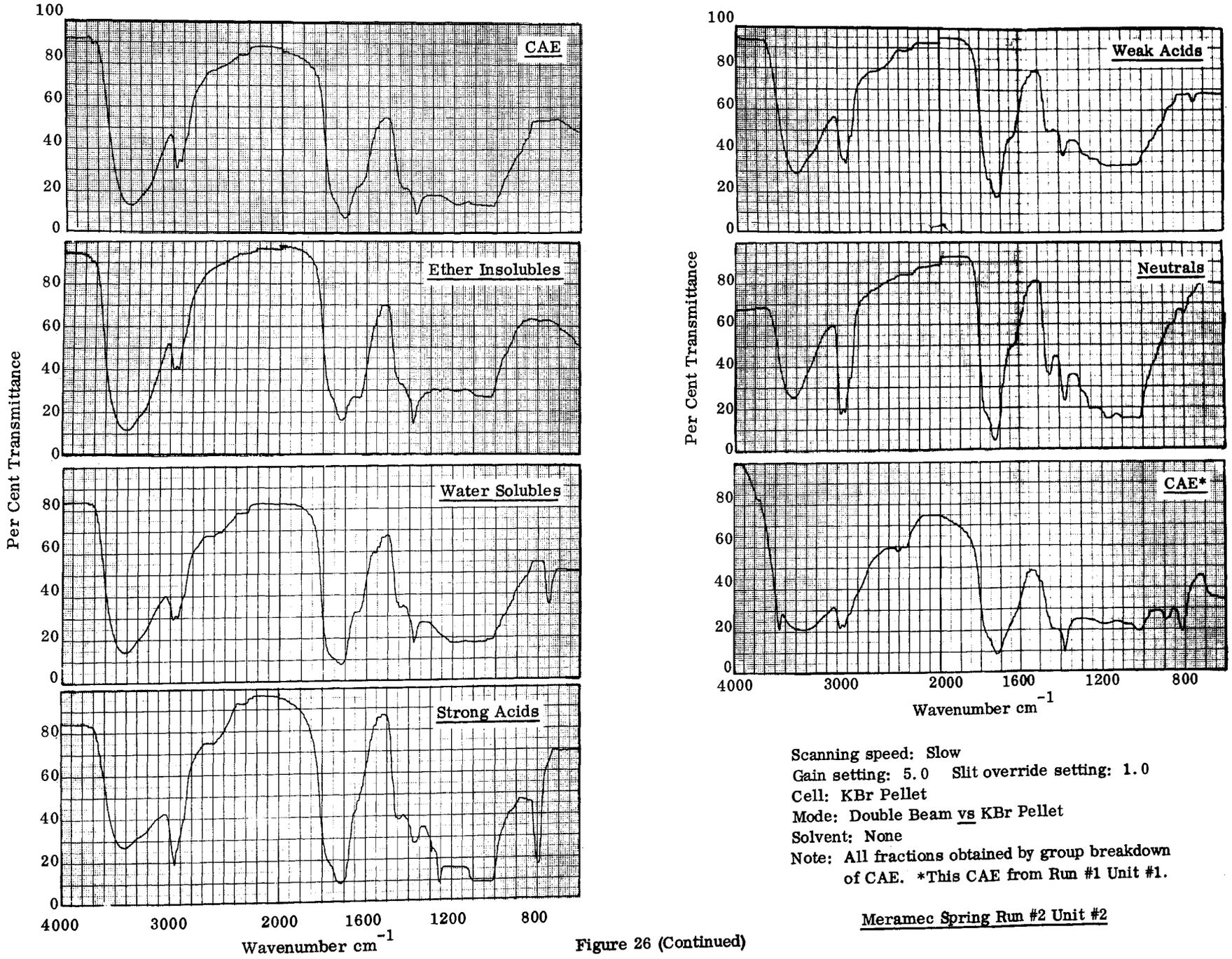


Scanning speed: Slow
Gain setting: 3.0 Slit override setting: 0.0
Cell: 0.1 mm NaCl liquid cell
Mode: Double Beam vs Chloroform
Solvent: Chloroform
Note: All fractions obtained by group breakdown of CCE

Meramec Spring Run #1 Unit #1

Figure 26

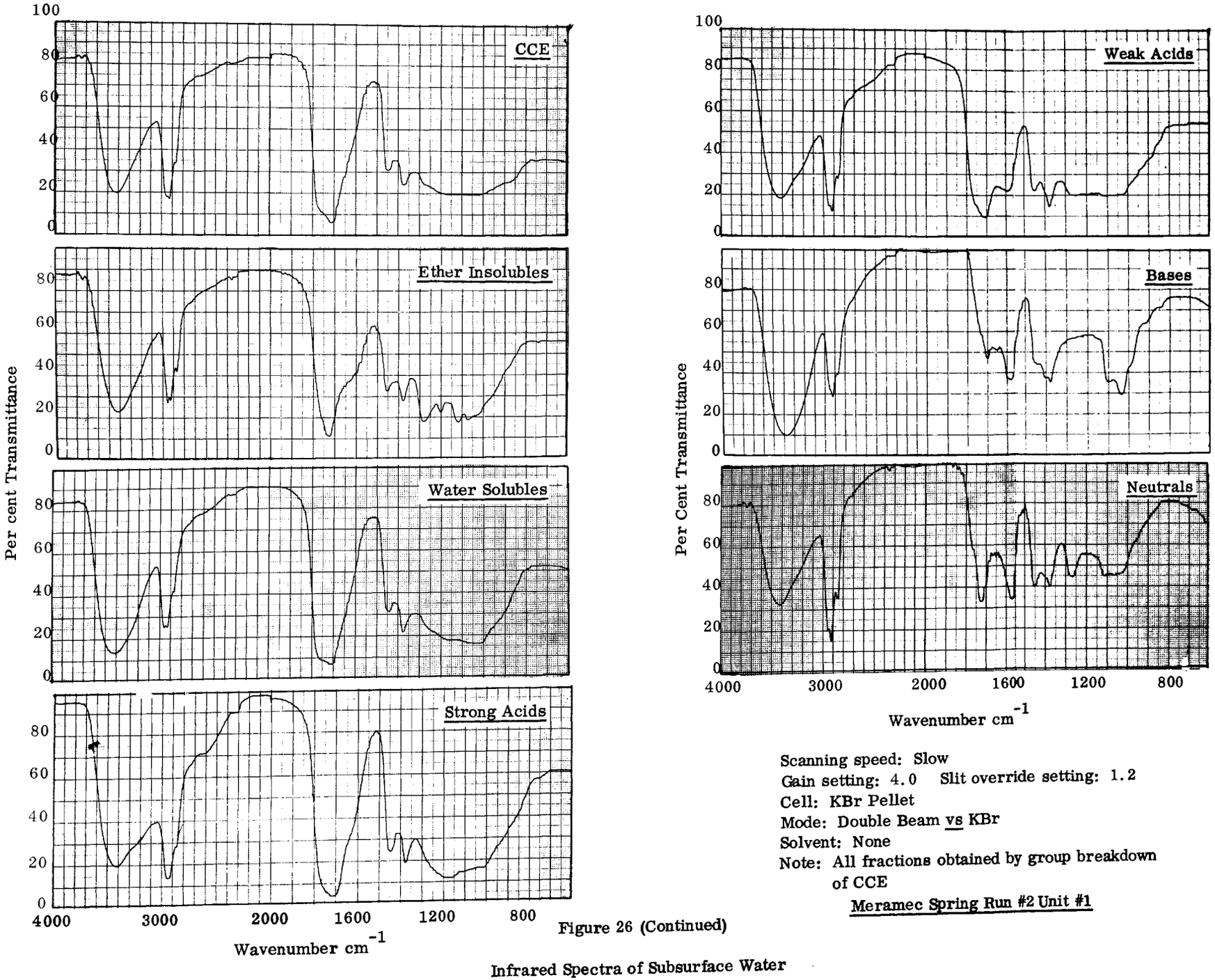
Infrared Spectra of Subsurface Water Organic Micropollutants



Scanning speed: Slow
Gain setting: 5.0 Slit override setting: 1.0
Cell: KBr Pellet
Mode: Double Beam vs KBr Pellet
Solvent: None
Note: All fractions obtained by group breakdown of CAE. *This CAE from Run #1 Unit #1.

Meramec Spring Run #2 Unit #2

Figure 26 (Continued)
Infrared Spectra of Subsurface Water Organic Micropollutants

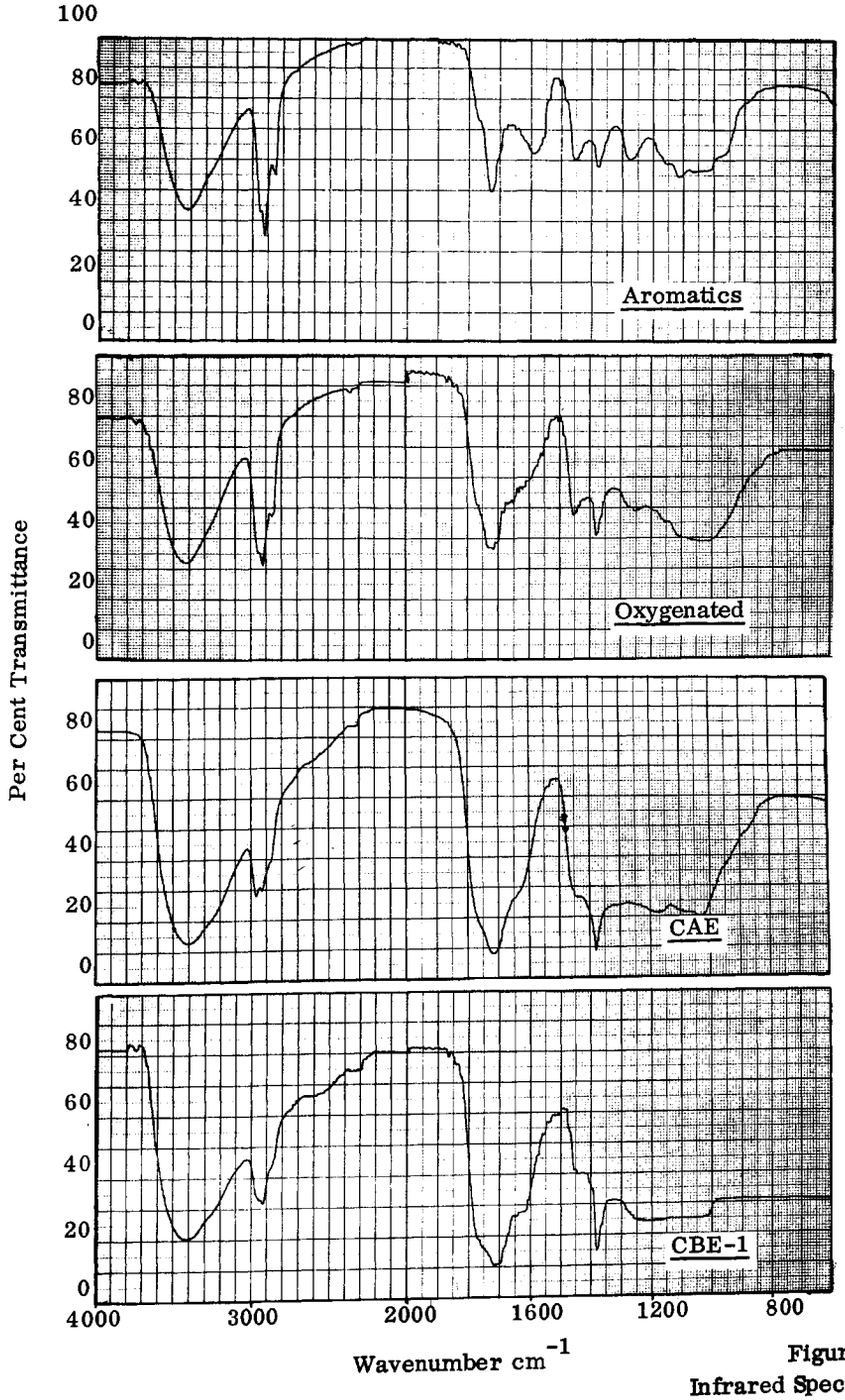


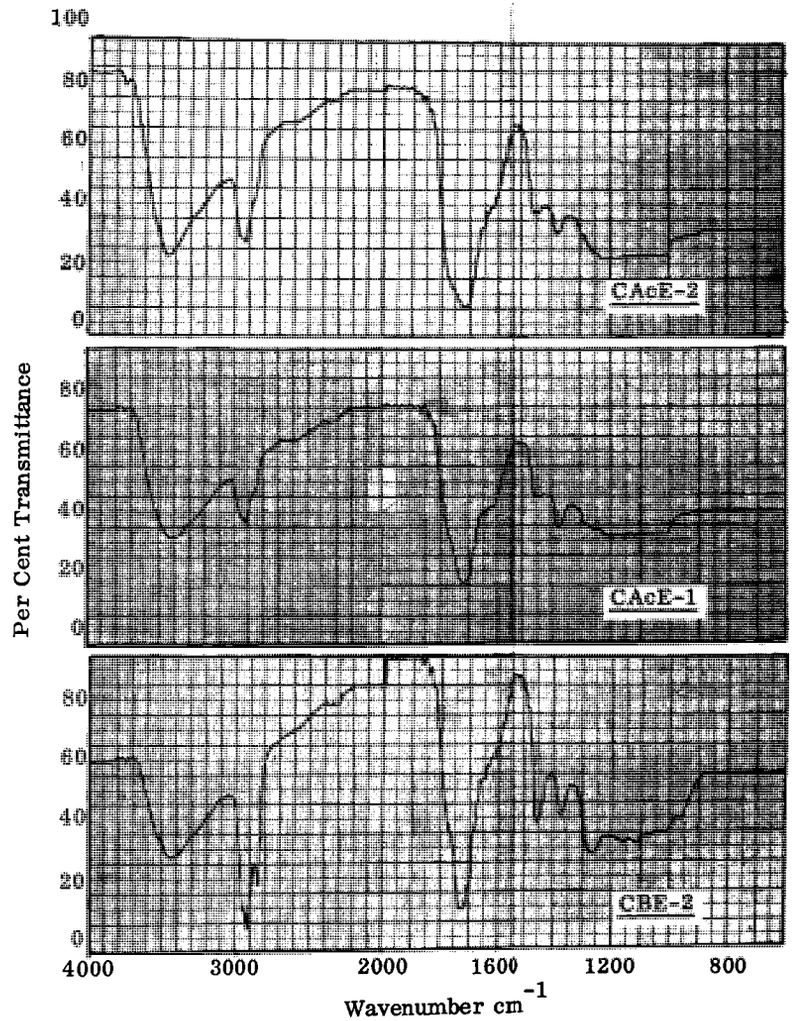
Scanning speed: Slow
Gain setting: 4.0 Slit override setting: 1.2
Cell: KBr Pellet
Mode: Double Beam vs KBr
Solvent: None
Note: All fractions obtained by group breakdown of CCE

Meramec Spring Run #2 Unit #1

Figure 26 (Continued)

Infrared Spectra of Subsurface Water
Organic Micropollutants

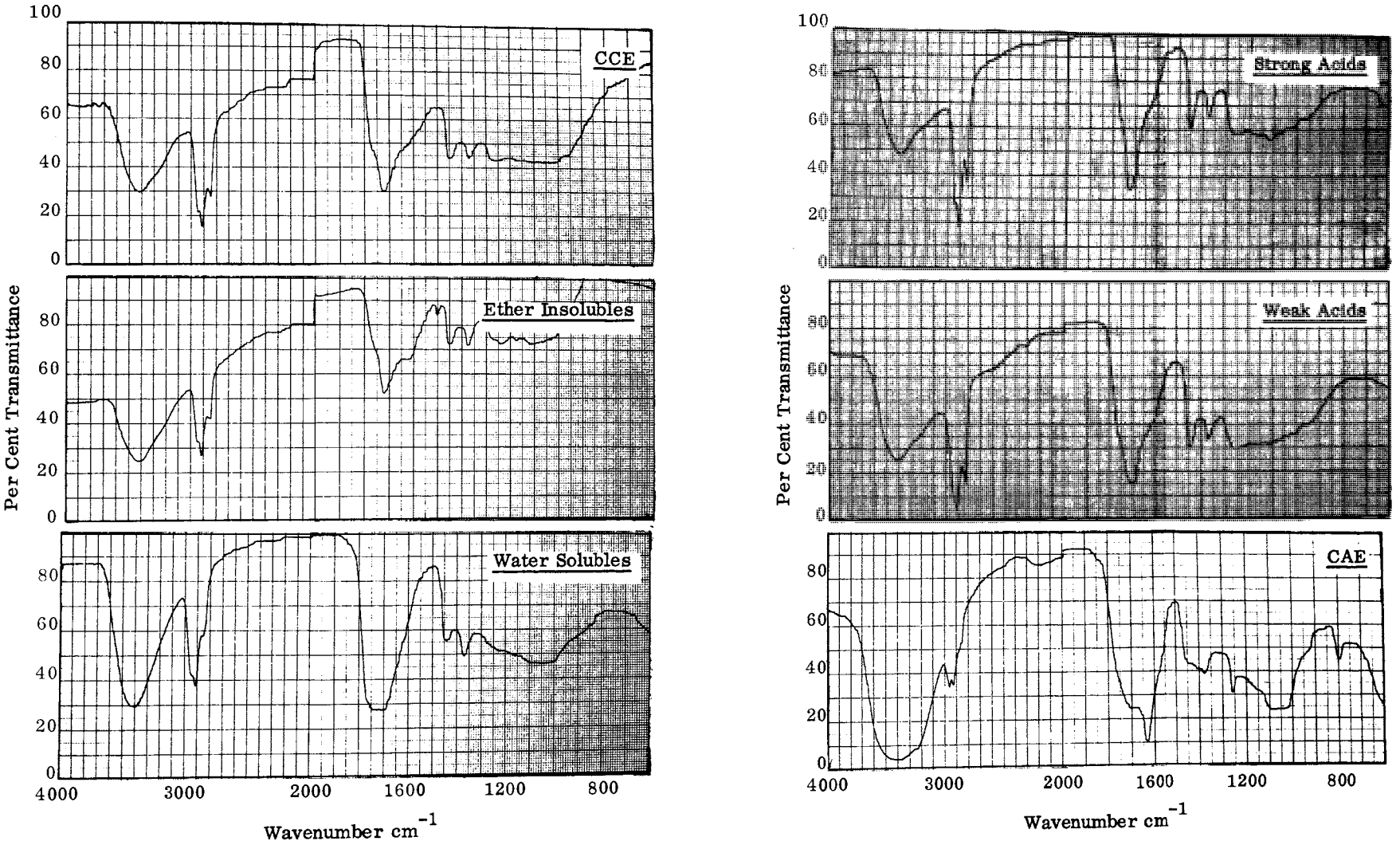




Scanning speed: Slow
Gain setting: 3.4 Slit override setting: 1.0
Cell: KBr Pellet
Mode: Double Beam vs KBr
Solvent: None
Note: All fractions obtained by group breakdown of CCE

Meramec Spring Run #2 Unit #1

Figure 26 (Continued)
Infrared Spectra of Subsurface Water Organic Micropollutants

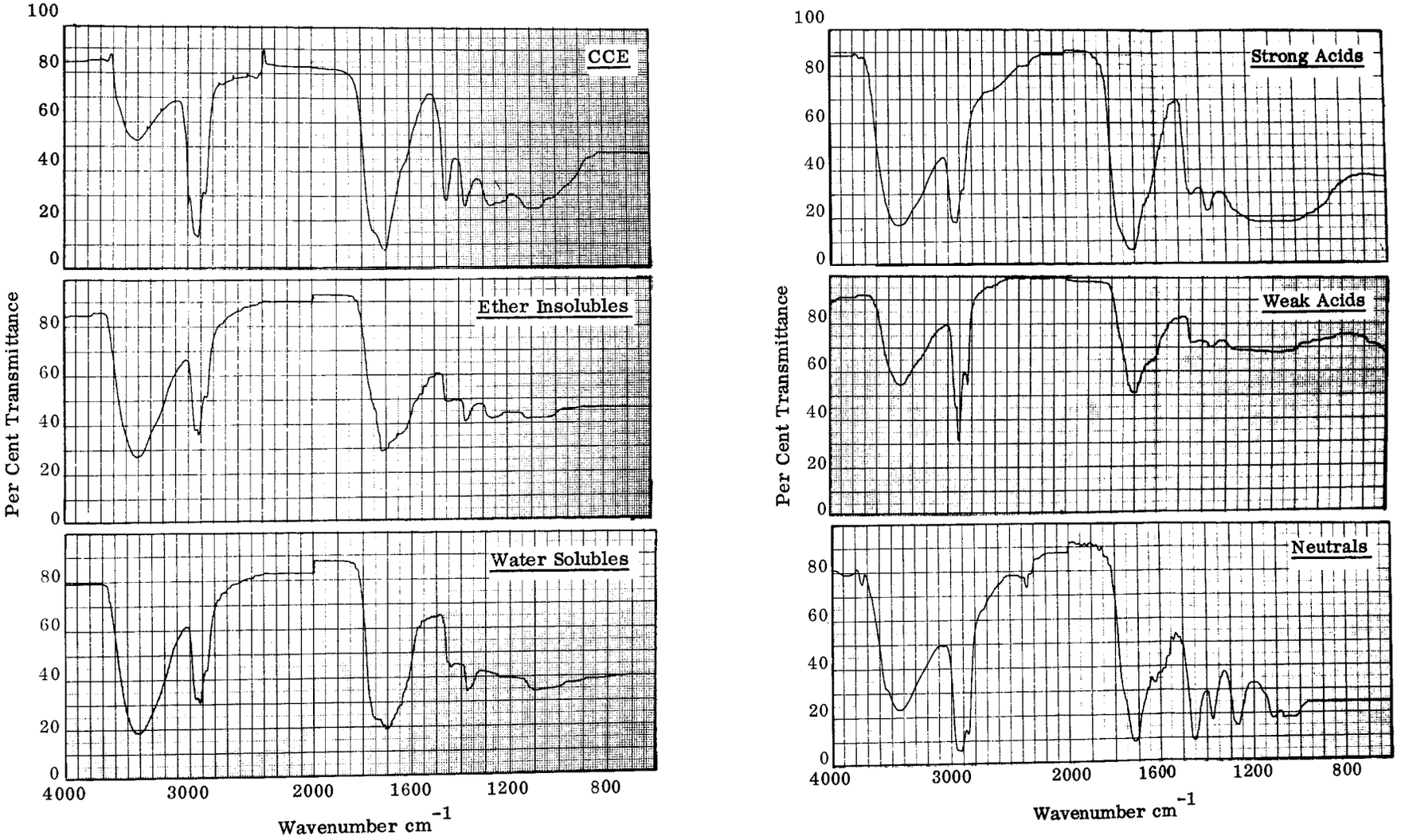


Scanning speed: Slow
Gain setting: 3.0 Slit override setting: 1.0
Cell: KBr Pellet
Mode: Double Beam vs KBr
Solvent: None
Note: All fractions obtained by group breakdown of CCE

Figure 26 (Continued)

UMR Well

Infrared Spectra of Subsurface Water
Organic Micropollutants



Scanning speed: Slow
Gain setting: 3.0 Slit override: 1.2
Cell: KBr Pellet
Mode: Double Beam vs KBr
Solvent: None
Note: All fractions obtained by group breakdown of CCE

Treated Missouri River Water

Figure 27

Infrared Spectra of Surface Water Organic Micropollutants

Table 25

Absorption Peaks Resulting from Infrared Analysis
Of Organic Micropollutants

Test Material	Solvent/ Cell	Absorption Peak Wavenumber cm^{-1}/intensity*/shape**													Probable Functional Groups
Meramec Spring Run #1 Unit #1															
CCE	Chloroform/ 0.1mm NaCl	3620/ m/sp	3420/ m/vb	2990/ vw/b	2930/ m/m	2830/ m/m	1710/ m/b		1445/ w/b	1370/ vw/m	1310/ vw/b	1215/ w/b	1000/ s/m		Aliphatic alcohol, aldehyde, carboxylic acid.
Ether Insolubles	Chloroform/ 0.1mm NaCl		3380/ mb/	2960/ s/m	2920/ vw/sh		1710/ m/b		1440/ w/m	1370/ w/m		1230/ vw/b	1037/ w/m		Aliphatic alcohol, carboxylic acid.
Water Solubles	Chloroform/ 0.1mm NaCl		3420/ m/b	2985/ vw/sh	2930/ m/m	2860/ vw/sh	1715/ s/b		1450/ w/m	1380/ m/m		1200/ vw/b	1010/ w/b		Aliphatic alcohol, aldehyde, carboxylic acid.
Strong Acids	Chloroform/ 0.1mm NaCl				2920/ m/s	2850/ w/sh	1710/ s/b	1505/ w/m	1415/ m/b	1370/ w/m					Aldehyde, carboxylic acid, aromatic ring.
Weak Acids	Chloroform/ 0.1mm NaCl				2920/ m/s	2840/ w/sh	1705/ s/b		1445/ m/b	1365/ w/b	1280 vw/b				Aliphatic aldehyde.
Neutrals	Chloroform/ 0.1mm NaCl		3400/ m/b		2910/ s/sp	2840/ m/sp	1715/ s/m		1450/ s/m	1365/ m/m		1150/ w/b			Aliphatic alcohol, aldehyde, carboxylic acid.
Oxygenated	Chloroform/ 0.1mm NaCl	3620/ m/sp	3440/ m/b	2980/ vw/sh	2930/ s/m	2820/ w/m	1710/ w/b	1590/ w/m	1445/ w/b				1000/ m/sp		Alcohol, aldehyde, carboxylic acid, aromatic ring.
CAE	None/ KBr Pellet	3560/ m/sp	3300/ w/vb	2970/ w/m	2930/ vw/m		1720/ s/b		1440/ w/sh	1381/ m/sp			1020/ vw/b	815/ m/m		Aliphatic alcohol, aldehyde, carboxylic acid.
Meramec Spring Run #2 Unit #2															
CAE	None/ KBr Pellet	3400/ s/vb	2970/ m/m	2930/ w/m		1715/ s/m	1630/ vw/sh		1440/ vw/sh	1376/ m/m			1010/ vw/sh		Alcohol, carboxylic acid, aromatic ring.
Ether Insolubles	None/ KBr Pellet	3400/ s/vb	2970/ m/m	2930/ vw/m		1715/ s/m	1630/ vw/sh			1382/ m/sp			1060/ vw/vb		Alcohol, carboxylic acid, aromatic ring.
Water Solubles	None/ KBr Pellet	3430/ s/b	2980/ m/m	2935/ vw/m		1720/ s/m			1440/ vw/m	1381/ m/sp		1120/ w/vb		750/ m/m	Aliphatic alcohol, carboxylic acid.
Strong Acids	None/ KBr Pellet	3420/ s/b	2960/ m/sp			1720/ s/b			1445/ vw/m	1370/ m/m	1255/ m/m		1055/ w/vb	795/ m/m	Aliphatic alcohol, carboxylic acid.
Weak Acids	None/ KBr Pellet	3420/ s/b	2960/ vw/sh	2933/ m/sp	2860/ vw/sh	1715/ s/m			1440/ vw/m	1380/ m/m		1100/ vw/vb		750/ w/m	Aliphatic alcohol, aldehyde, carboxylic acid.
Neutrals	None/ KBr Pellet	3430/ s/b	2970/ m/m	2930/ w/m	2862/ vw/sh	1725/ s/m			1445/ m/m	1370/ m/m	1255/ vw/sh	1170/ w/vb	1060/ vw/vb	795/ vw/m	Aliphatic alcohol, aldehyde, carboxylic acid.

*intensity: s - strong (>35%); m - medium (10-35%); w - weak (2-10%); vw - very weak (<2%) (75).
**shape at peak maximum: sp - sharp (pen width); m - medium (pen width to 10 cm^{-1}); b - broad (10-20 cm^{-1}); vb - very broad (>20 cm^{-1}); sh - shoulder band (75).

Table 25 (Continued)

Absorption Peaks Resulting from Infrared Analysis
Of Organic Micropollutants

Test Material	Solvent/ Cell	Absorption Peaks Wavenumber cm^{-1}/intensity*/shape**													Probable Functional Groups
Meramec Spring Run #2 Unit #1															
CCE	None/ KBr Pellet	3420/ s/vb	2960/ vw/sh	2925/ s/m	2860/ vw/sh		1712/ s/m		1450/ s/m	1370/ m/m			1100/ m/vb			Aliphatic alcohol, aldehyde, carboxylic acid.
Ether Insolubles	None/ KBr Pellet	3420/ s/vb	2955/ s/sp	2925/ w/sp	2865/ vw/sp		1725/ s/m		1455/ m/m	1376/ m/m	1280/ m/m	1200/ w/m	1120/ w/m	1075/ vw/m		Aliphatic alcohol, aldehyde, carboxylic acid.
Water Solubles	None/ KBr Pellet	3420/ s/vb	2940/ m/vb		2870/ vw/sh		1710/ s/vb		1445/ s/m	1375/ m/m					1030/ m/vb	Aliphatic alcohol, aldehyde, carboxylic acid.
Strong Acids	None/ KBr Pellet	3420/ s/vb	2940/ m/vb		2860/ vw/sh		1715/ s/m		1445/ s/m	1378/ m/m		1180/ m/vb				Aliphatic alcohol, aldehyde, carboxylic acid.
Weak Acids	None/ KBr Pellet	3410/ s/vb	2950/ vw/sh	2920/ m/sp	2860/ vw/sh	1720/ vw/sh	1705/ s/m	1580/ vw/m	1450/ m/m	1380/ m/m		1160/ w/vb				Alcohol, aldehyde, carboxylic acid, aromatic ring.
Bases	None/ KBr Pellet	3380/ s/vb		2920/ m/sp	2870/ vw/sh		1700/ s/m	1570/ m/b	1455/ vw/sh	1380/ m/m			1100/ vw/sh		1035/ m/m	Alcohol, aldehyde, carboxylic acid, aromatic ring.
Neutrals	None/ KBr Pellet	3420/ s/vb	2950/ vw/sh	2920/ s/sp	2850/ w/m		1710/ s/m	1570/ m/m	1455/ w/m	1375/ w/m	1270/ m/m			1085/ m/vb		Alcohol, aldehyde, carboxylic acid, aromatic ring.
Aromatics	None/ KBr Pellet	3420/ s/vb	2950/ vw/sh	2920/ s/sp	2860/ vw/m		1720/ s/m		1450/ m/m	1380/ w/m	1250/ vw/m				1030/ m/vb	Aliphatic alcohol, aldehyde, carboxylic acid.
Oxygenated	None/ KBr Pellet	3430/ s/vb	2950/ vw/sh	2920/ s/sp	2850/ w/sp		1725/ s/m	1595/ w/b	1450/ m/m	1378/ w/m	1275/ m/b		1110/ w/m		1010/ vw/sh	Alcohol, aldehyde, carboxylic acid, aromatic ring.
CAE	None/ KBr Pellet	3400/ s/b	2962/ m/m	2925/ vw/m			1715/ s/m		1450/ vw/sh	1380/ m/sp		1180/ vw/vb			1030/ vw/vb	Aliphatic alcohol, carboxylic acid.
CBE-1	None/ KBr Pellet	3410/ s/b		2920/ m/m		1730/ vw/sh	1710/ s/b		1450/ vw/sh	1380/ m/sp			1120/ w/vb			Aliphatic alcohol, carboxylic acid.
CAcE-2	None/ KBr Pellet	3420/ s/b		2920/ m/m			1718/ s/m		1455/ vw/sh	1380/ m/m			1100/ w/vb			Aliphatic alcohol, carboxylic acid.
CAcE-1	None/ KBr Pellet	3440/ s/m	2950/ vw/sh	2920/ s/sp	2850/ w/sp		1725/ s/m		1460/ s/m	1377/ m/m	1270/ m/m				1000/ vw/sh	Aliphatic alcohol, aldehyde, carboxylic acid.
CBE-2	None/ KBr Pellet	3450/ s/m	2980/ vw/sh	2930/ m/m			1720/ s/m		1450/ w/b	1380/ m/m			1120/ w/vb			Aliphatic alcohol, carboxylic acid.

*intensity: s - strong (>35%); m - medium (10-35%); w - weak (2-10%); vw - very weak (<2%) (75).
**shape at peak maximum: sp - sharp (pen width); m - medium (pen width to 10 cm^{-1}); b - broad (10-20 cm^{-1}); vb - very broad (>20 cm^{-1}); sh - shoulder bands (75).

Table 25 (Continued)

Absorption Peaks Resulting from Infrared Analysis
Of Organic Micropollutants

Test Material	Solvent/ Cell	Absorption Peaks Wavenumber cm^{-1}/intensity*/shape**													Probable Functional Groups
UMR Well Unit #1															
CCE	None/ KBr Pellet		3415/ m/b	2950/ vw/sh	2915/ s/sp	2845/ w/sp	1722/ s/m		1450/ m/b	1370/ w/b					Aliphatic alcohol, aldehyde, carboxylic acid.
Ether Insolubles	None/ KBr Pellet		3410/ m/b	2950/ vw/sh	2915/ s/sp	2845/ vw/sh	1722/ s/m	1500/ w/m	1450/ m/m	1375/ w/m		1235/ vw/b			Alcohol, aldehyde, carboxylic acid, aromatic ring.
Water Solubles	None/ KBr Pellet		3420/ s/b	2950/ vw/sh	2915/ s/sp	2850/ vw/sh	1720/ s/vb		1440/ m/b	1370/ m/m					Aliphatic alcohol, aldehyde, carboxylic acid.
Strong Acids	None/ KBr Pellet		3420/ s/b	2950/ vw/sh	2915/ s/sp	2845/ m/sp	1720/ s/b		1450/ s/m	1370/ m/m			1115/ vw/vb		Aliphatic alcohol, aldehyde, carboxylic acid.
Weak Acids	None/ KBr Pellet		3410/ s/vb	2940/ vw/sh	2905/ s/sp	2835/ m/sp	1705/ s/b		1445/ s/b	1370/ m/m		1250/ vw/vb			Aliphatic alcohol, aldehyde, carboxylic acid.
CAE	None/ KBr Pellet		3390/ s/vb	2960/ m/m	2921/ w/m		1710/ vw/sh	1630/ s/m	1445/ vw/sh	1375/ vw/m	1260/ m/m			1050/ m/vb	795/ m/m	Alcohol, carboxylic acid, aromatic ring.
Missouri River at St. Louis															
CCE	None/ KBr Pellet		3420/ m/vb	2960/ vw/sh	2930/ s/m	2870/ vw/sh	1700/ s/m		1450/ m/m	1358/ m/m		1200/ m/vb	1080/ w/vb		Aliphatic alcohol, aldehyde, carboxylic acid.
Ether Insolubles	None/ KBr Pellet		3400/ s/m	2950/ w/m	2920/ m/m	2855/ vw/sh	1719/ s/m		1450/ vw/sh	1375/ w/m	1260/ m/b		1080/ w/vb		Aliphatic alcohol, aldehyde, carboxylic acid.
Water Solubles	None/ KBr Pellet		3420/ s/vb	2950/ vw/m	2920/ m/m	2860/ vw/sh	1700/ s/m		1440/ vw/m	1378/ m/m		1200/ vw/vb			Aliphatic alcohol, aldehyde, carboxylic acid.
Strong Acids	None/ KBr Pellet		3420/ s/vb	2950/ vw/m	2920/ m/m	2850/ vw/sh	1720/ s/m		1440/ m/m	1380/ m/m					Aliphatic alcohol, aldehyde, carboxylic acid.
Weak Acids	None/ KBr Pellet		3420/ s/vb	2950/ vw/m	2920/ m/m	2860/ vw/sh	1710/ s/m		1445/ m/m	1380/ m/m					Aliphatic alcohol, aldehyde, carboxylic acid.
Neutrals	None/ KBr Pellet	3440/ s/vb		2950/ vw/sh	2920/ s/m	2860/ w/m	1715/ s/m		1450/ m/m	1375/ m/m	1270/ m/m		1115/ w/b	1045/ w/vb	Aliphatic alcohol, aldehyde, carboxylic acid.

*intensity: s - strong (>35%); m - medium (10-35%); w - weak (2-10%); vw - very weak (<2%) (75).
**shape at peak maximum: sp - sharp (pen width); m - medium (pen width to 10 cm^{-1}); b - broad (10-20 cm^{-1}); vb - very broad (>20 cm^{-1}); sh - shoulder bands (75).

organic micropollutants and indicate the general character of the trace organics. Most of the spectra exhibited absorption bands indicative of the following groups:

Absorption Range	Group Indicated
3500 - 3400 cm^{-1}	Aliphatic hydroxyl group
2990 - 2900 cm^{-1}	Aliphatic hydrogen
2860 - 2850 cm^{-1}	Aldehyde hydrogen
1730 - 1710 cm^{-1}	Carbonyl group
1450 - 1380 cm^{-1}	Aliphatic hydrogen

Some of the samples exhibited absorption peaks at 1570, 1180 and 1100 to 1010 cm^{-1}, all of which would represent aromatic groups. These data would, therefore, indicate that the majority of the organic micropollutants were aliphatic alcohols, aldehydes, and carboxylic acids, with some trace organics having aromatic groups.

C. IDENTIFICATION STUDIES

The gas chromatographic studies demonstrated by the number of chromatogram peaks obtained for each material that the organic micropollutants and even their group breakdown fractions were composed of many compounds; however, the possibility that some of the peaks could have resulted from pyrolysis of the sample should not be disregarded. Infrared analysis provided an indication of the nature of the organics but was not capable of positive identification of these complex materials. A more efficient method of separating the trace organics into less complex components was therefore necessary. Because of its very powerful separating ability, gas chromatography was evaluated as a fractionating device preceding infrared analysis. This necessitated the use of

a system to collect fractions corresponding to individual chromatogram peaks as they were being eluted from the column.

Since the hydrogen flame detector which was employed in these studies destroyed the sample as it passed through the flame, an effluent splitter was installed between the column exit and the detector entrance to bypass a given percentage of the sample and allow it to be collected. The effluent splitter divided the effluent from the column into two parts; one part going to the detector and one part going to an exhaust port where it could be collected. A variable effluent splitter was employed and permitted split ratios ranging from 1 to 1 up to 1 to 30 (1 part going to the detector, 30 parts going to the collection device). A ratio of 1 to 15 was employed for the separation and collection of the organics. Collection of the components of the trace organics was accomplished with a Beckman GC-IR fraction collection system* which is shown in Figure 28. This system consisted of a 3 foot variable temperature heated line (1/16 inch inside diameter) terminating in a collection head with a heated needle, a 12 volt variable current power supply, and a silver chloride infrared cell** which was fitted over the heated needle. The heated line was attached to the exit port of the gas chromatograph with a silicone elastomer septum. Temperature was varied by adjusting the current through the line which caused a power loss and resulted in the release of heat. The components of the trace organics separated by the chromatograph were taken in the vapor state from the

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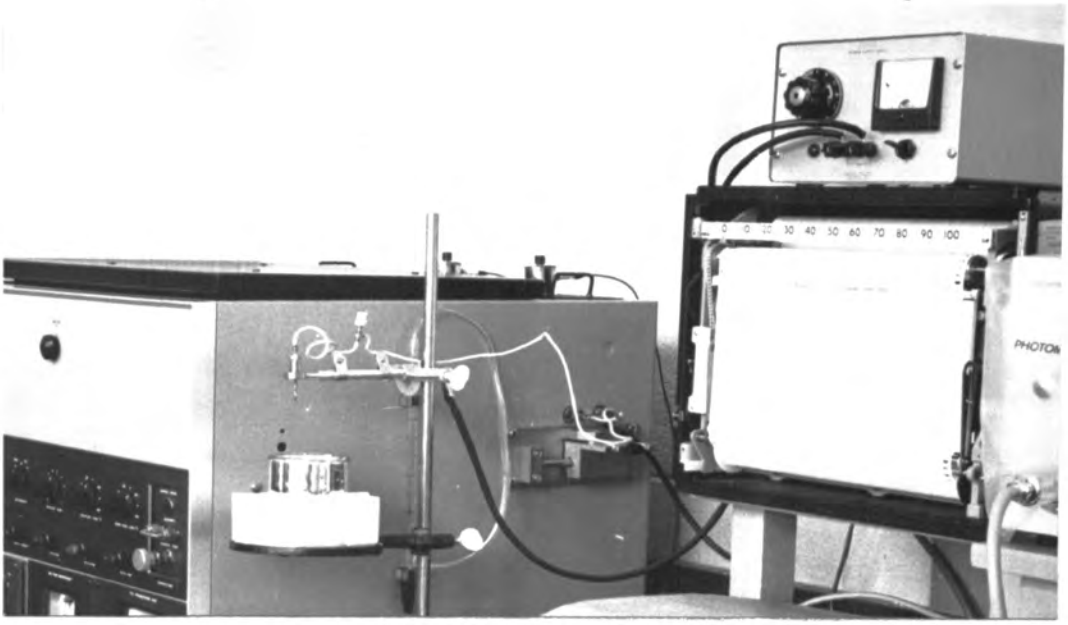
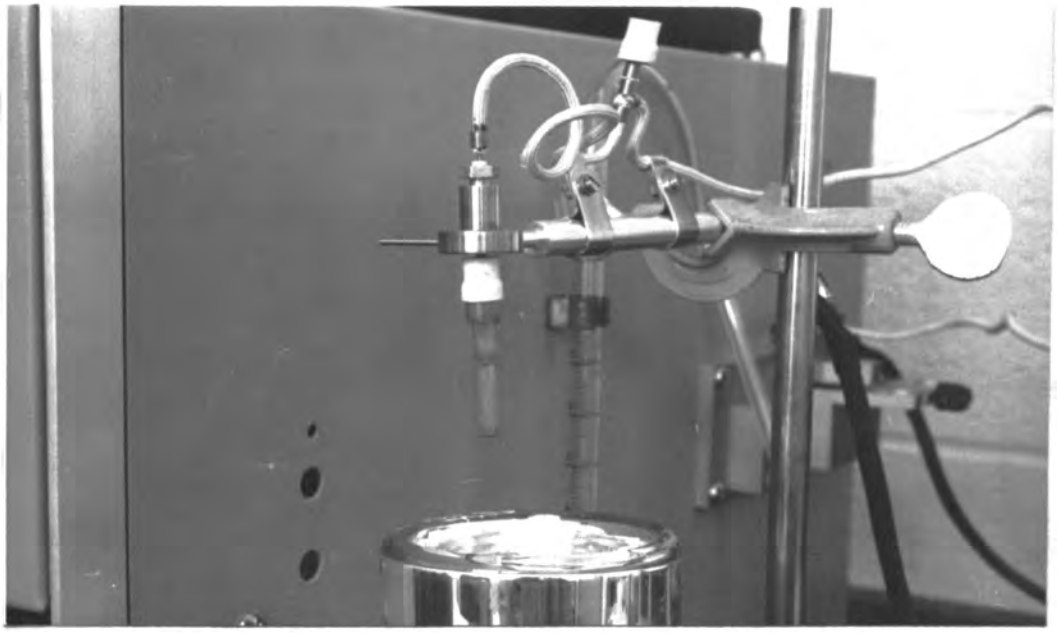
*A product of Beckman Instruments, Inc., Fullerton, Calif.
**Extrocell, a product of Beckman Instruments, Inc., Fullerton, Calif.



Overall Collection Arrangement



Close-up of Heated Needle and Extrocell

Figure 28

Beckman GC-IR Fraction Collection System

exhaust port to the heated needle, where they were condensed in a silver chloride Extrocell immersed in an acetone-ice-salt mixture. After the component had been collected, a volume of solvent equal to the volume of the cell window was added, and the cell was centrifuged with an International clinical centrifuge* for two minutes to force the sample into the cell window. The cell was then subjected to infrared analysis, and the resulting spectrum was considered to correspond to a particular component. A 1/4 inch diameter, 5 foot column and a 50 μl sample volume were employed with the chromatograph which functioned as a preparative instrument. After each separation, the heated line was flushed with solvent to assure that no accumulation of materials which may not have passed completely through the line occurred. It should be mentioned that an attempt was also made to collect the fractions from the gas chromatograph in potassium bromide and analyze the material in pellet form. This method was not used, however, because of the larger quantity of sample required to obtain a spectrum.

The collection system and Extrocells of two different pathlengths (0.1 and 0.01 mm) were evaluated using methyl hexanoate, methyl octanoate, and methyl dodecanoate; all of these substances were methyl esters of fatty acids and liquids at room temperature. A 50 μl volume of a 10 per cent solution of the methyl esters in methanol was injected into a column containing 15 per cent FFAP on 60/80 mesh Chromosorb W, and the total effluent sample was

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*Model CL, a product of the International Equipment Co., Needham Heights, Mass.

collected in the Extrocell. The amount of FFAP was increased to 15 per cent from the 10 per cent which was used for analytical purposes to permit the application of larger quantities of organics to the column. The gas chromatograph was operated isothermally at 100°C for 12 minutes. The flow rates for the nitrogen, hydrogen, and air were 50, 40, and 250 ml per minute, respectively. The Extrocell containing the effluent sample was centrifuged and used to determine the infrared spectrum of the sample. Because the small window area of the cell reduced the total amount of energy passing through it, the manual slit override was employed to increase the slit width and, accordingly, the amount of energy in the system. This procedure was employed with the individual methyl esters and an equal volume mixture of the three compounds. A second sample of this mixture was injected into the chromatograph; however, in this case, the first peak (methyl hexanoate) was collected in one cell and the remaining two peaks were collected in another cell. The corresponding gas chromatogram is shown in Figure 29; infrared spectra for the fractions collected are presented in Figure 30 together with corresponding spectra obtained directly on the infrared analyzer. The spectra shown in Figure 30 are for the 0.1 mm pathlength Extrocells; however, equally good spectra were obtained with the 0.01 mm cells. Spectra of the samples collected from the gas chromatograph when the individual methyl esters were used were essentially identical to those obtained by direct infrared analysis and, consequently, are not presented. The results of this evaluation study would indicate that it was possible to collect the fractions of a mixture separated with the gas chromatograph and to obtain spectra which were very similar to the spectra of the pure compounds.

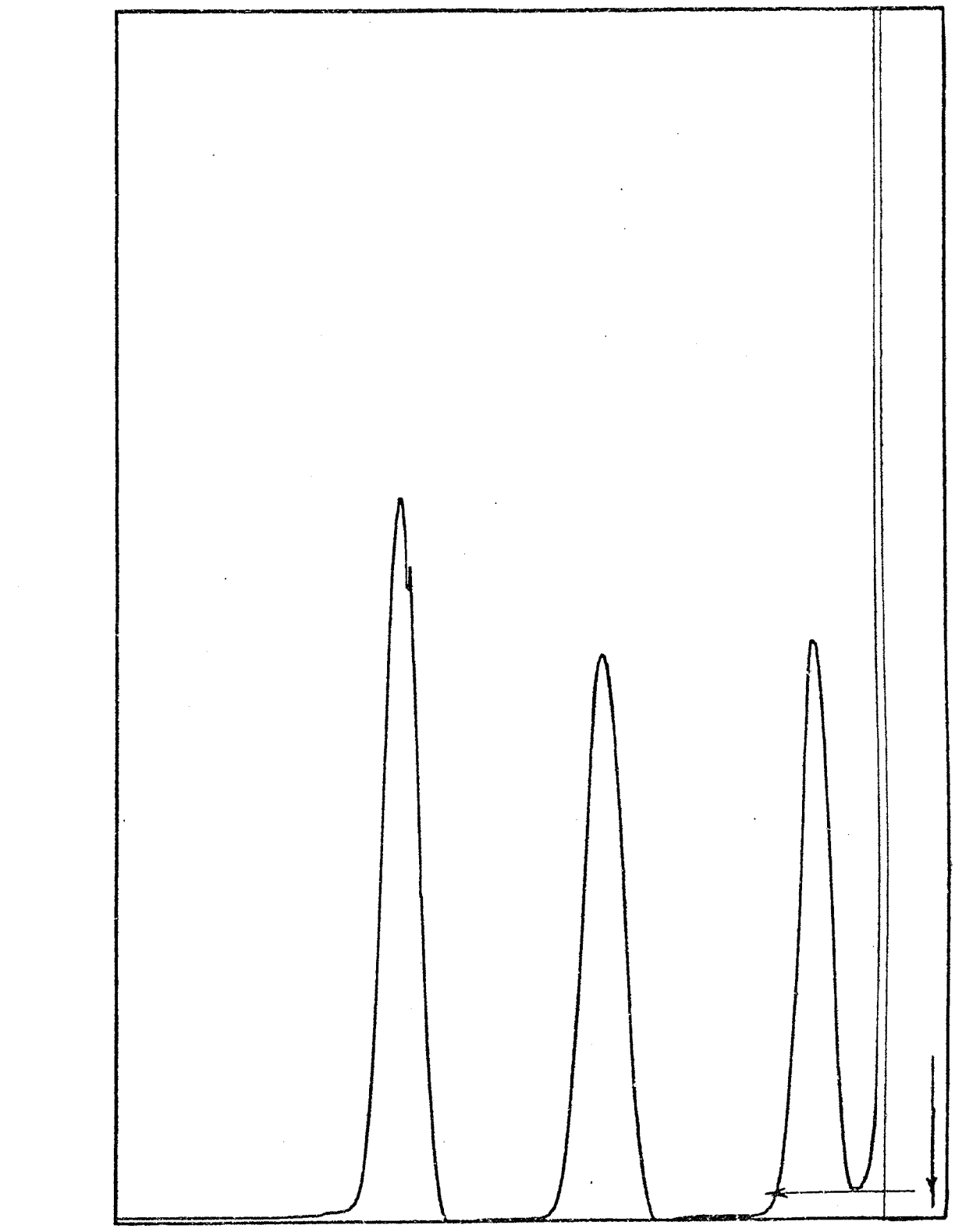
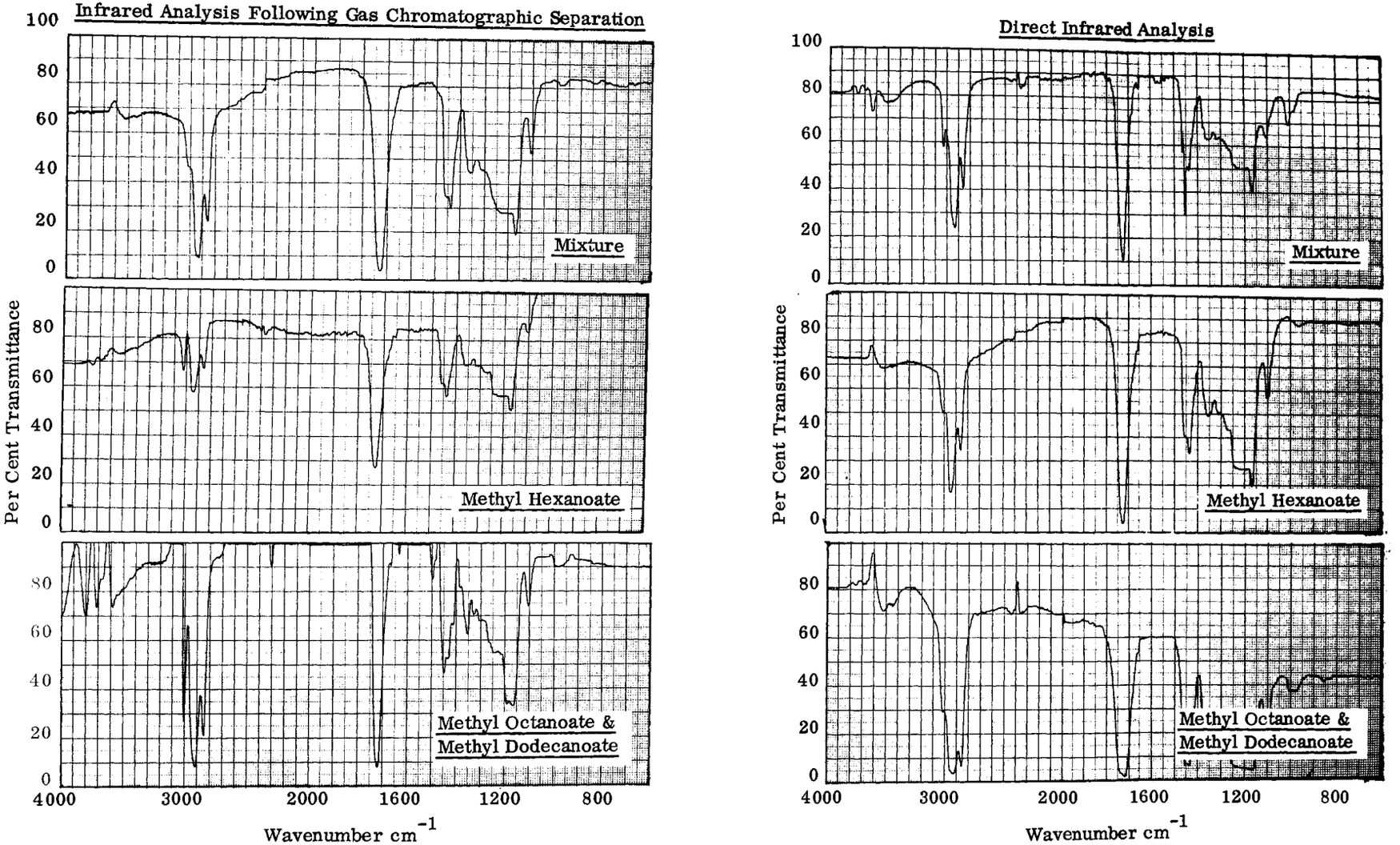


Figure 29

Gas Chromatogram of Methyl Esters of Fatty Acids
With 15% FFAP



Column: 15% FFAP on 60/80 Chromosorb W
Cell: 0.1 mm AgCl
Solvent: Chloroform
Mode: Double Beam vs Chloroform
Gain setting: 4.0 Slit override: 1.2
Scanning speed: Slow

Mixture of Methyl Hexanoate, Octanoate, and Dodecanoate (1:1:1 by volume)

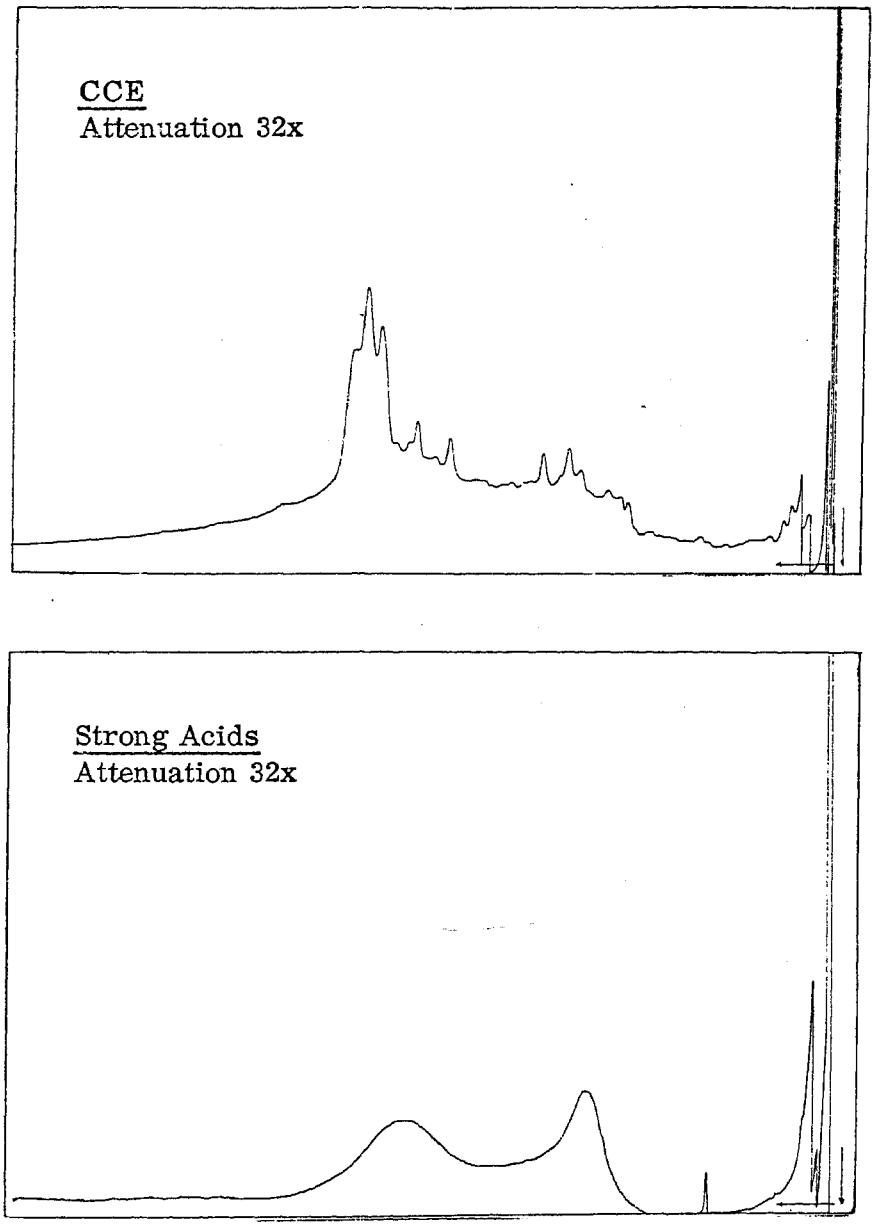
Figure 30

Infrared Spectra of Methyl Esters of Fatty Acids

When the collection of the trace organics was attempted using the 0.01 mm pathlength Extrocell, difficulty was encountered in forcing with chloroform enough material into the cell window to be detected by the infrared spectrophotometer. Even when samples of the organics were placed directly above the cell window, the material could not be forced into the small opening and only the solvent entered the cell as evidenced by the resulting infrared spectra. The use of other solvents and higher speed centrifugation did not overcome this difficulty. Larger pathlength (0.1 mm) cells were obtained and their wider window opening permitted collection of the trace organics.

The Meramec Spring Run #2 Unit #1 CCE and its corresponding strong acid group breakdown fraction, and the treated Missouri River water CCE and its neutral fraction were subjected to gas chromatographic and infrared analysis, and the resulting chromatograms and spectra are presented in Figures 31 through 34. In Figures 32 and 34, spectra of the corresponding compounds obtained by direct infrared analysis are also presented to facilitate comparison. It should be pointed out that strong absorption peaks above 3500 cm^{-1}, at 3000 and 2390 cm^{-1}, and between 1600 and 1500 cm^{-1} were observed and attributed to interference caused by the Extrocells employed in the analysis. Although an effort was made to match these silver chloride cells as closely as possible using chloroform before collection, it was not found possible to eliminate the presence of the fringe bands.

It may be seen from the spectra presented in Figures 32 and 34 that the absorption band at 3400 cm^{-1} was missing from all the samples collected from the gas chromatograph. This peak corresponded to a hydroxyl group which



Column: 15% FFAP on 60/80 Chromosorb W
Conditions: Iso. @ 75°C for 2 min.; LTP to 255°C @ 10°C per minute; Iso. @ 255°C for 10 min.

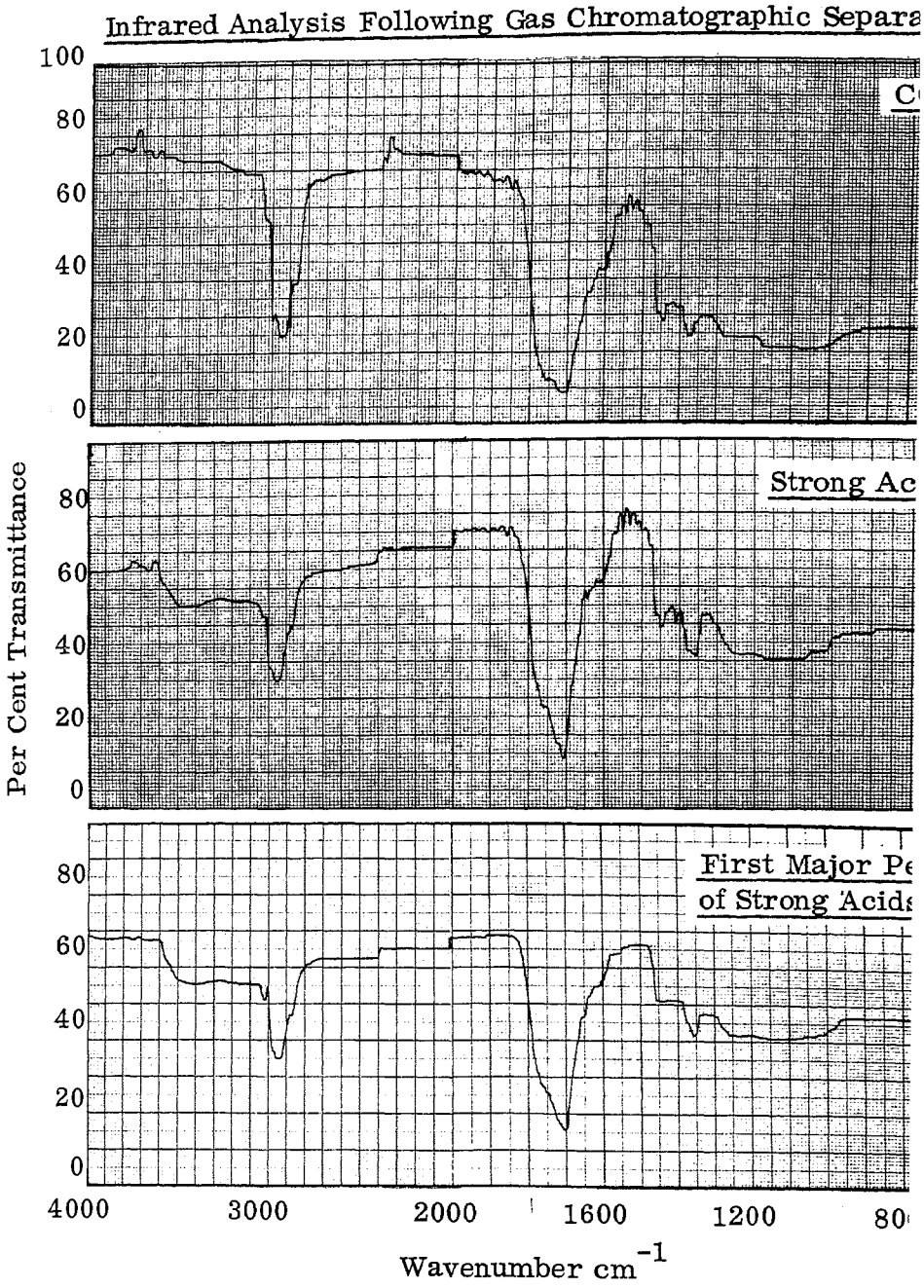
6 4 2 0

Scale: 1" = 6.7 minutes

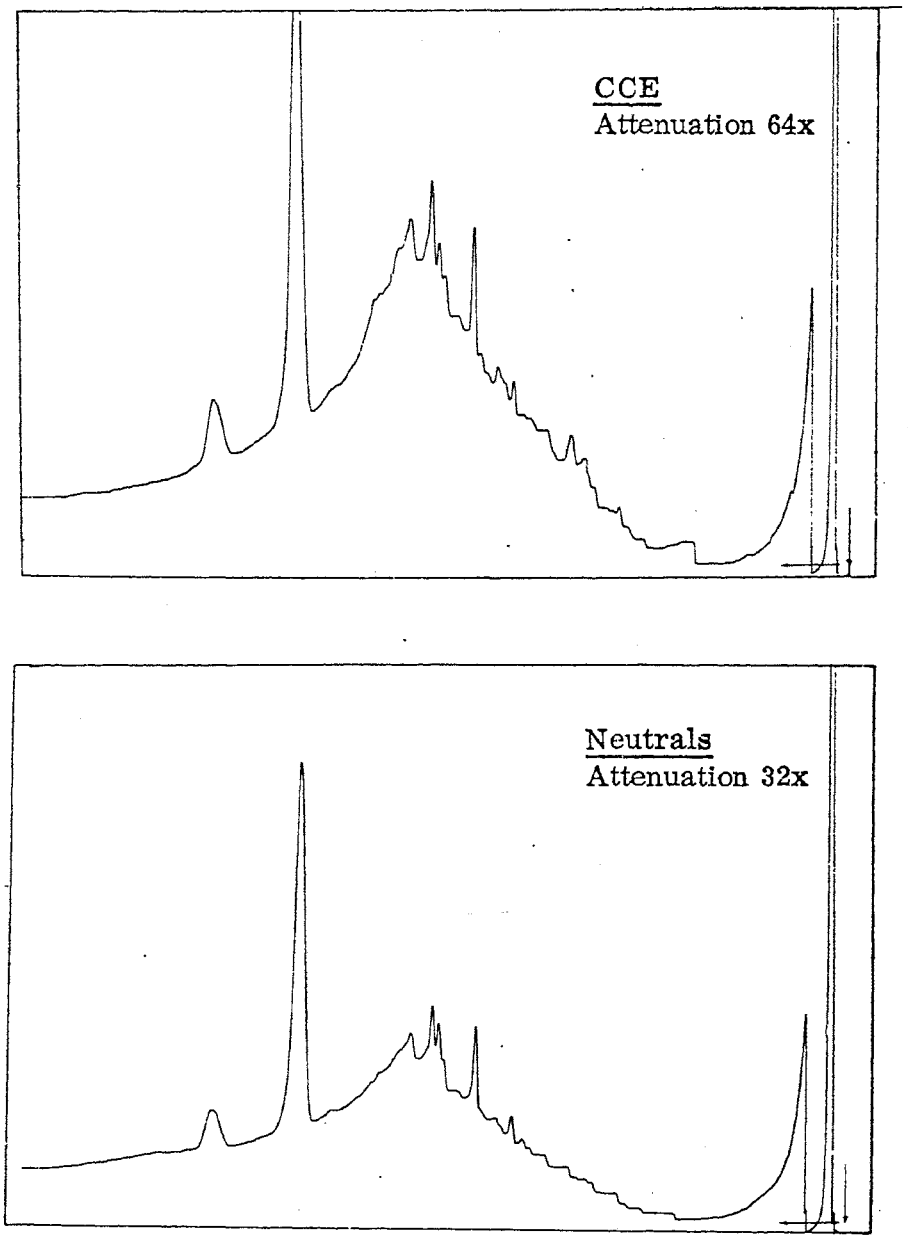
Meramec Spring Run #2 Unit #1

Figure 31

Gas Chromatograms Used in the Identification Of Subsurface Water Organic Micropollutants



Infrare
Of Subsu



Column: 15% FFAP on 60/80 Chromosorb W
Conditions: Iso. @ 75°C for 2 min.; LTP to 255°C
@ 10°C per min.; Iso. for 10 min.
Chart speed: 30 inches per hour

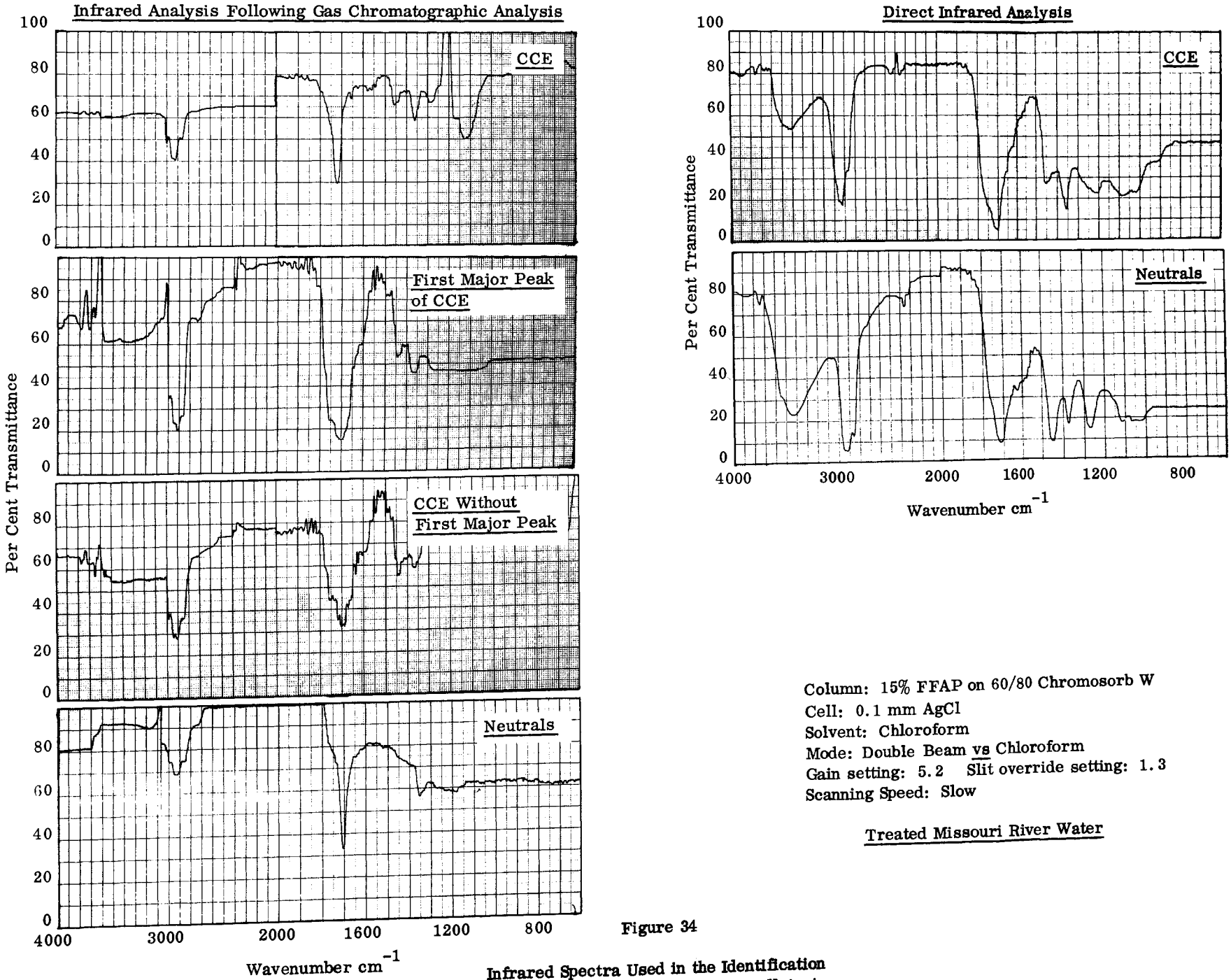
6 4 2 0

Scale: 1" = 6.7 minutes

Treated Missouri River Water

Figure 33

Gas Chromatograms Used in the Identification
Of Surface Water Organic Micropollutants



Column: 15% FFAP on 60/80 Chromosorb W
Cell: 0.1 mm AgCl
Solvent: Chloroform
Mode: Double Beam vs Chloroform
Gain setting: 5.2 Slit override setting: 1.3
Scanning Speed: Slow

Treated Missouri River Water

Figure 34

Infrared Spectra Used in the Identification Of Surface Water Organic Micropollutants

apparently was either retained on the column or modified during the chromatographic separation. In addition, it was observed that the same absorption bands were present in the spectra of the collected samples containing only one CCE peak, all the remaining CCE peaks, and the total CCE material recovered from either subsurface or surface water. This was also true for the subsurface water strong acid and the surface water neutral group breakdown fractions. If it may be assumed that the individual chromatogram peaks collected from the subsurface water CCE and the surface water strong acids fraction represented individual compounds, then the presence of the same major absorption bands in the infrared spectra of the individual peaks, group breakdown fractions, and parent materials would seem to indicate that these organics are composed of homologous compounds.

The absorption peaks in the infrared spectra of the collected samples indicated that these compounds contained aliphatic hydrogens (2900, 2850, 1450, and 1380 cm^{-1}) and carbonyl (1710 cm^{-1}) groups. However, the infrared spectra of the total surface water CCE and its neutral fraction and the subsurface water CCE and its strong acid fraction which were obtained without prior chromatographic analysis exhibited absorption bands characteristic of aliphatic hydrogen (2900, 2850, 1450, and 1380 cm^{-1}), carbonyl (1710 - 1700 cm^{-1}), and hydroxyl (3400 cm^{-1}) groups, indicating the possible presence of alcohol, aldehyde, and carboxylic acid functional groups. Because of the normally high vaporization temperature of the carboxylic acids, these materials may not have been eluted from the gas chromatograph. This is evidenced by the fact that no hydroxyl groups were found in the collected samples.

The GC-IR fraction collection system evaluated in this study with both a mixture of known methyl esters of fatty acids and with trace organics was found to be capable of separating and collecting small quantities of both simple and complex materials from the gas chromatograph which could then be subjected to infrared analysis. Of particular importance is the fact that it was possible to employ successfully this collection system with the organic micropollutants whose highly viscous to solid nature made the transfer of the material into the window opening of the cell difficult. Even when a quantity as small as one mg of organics was injected into the gas chromatograph, it was possible with the microcell (Extrocell) to obtain usable spectra even from the individual chromatogram peaks. Although the gas chromatographic studies had demonstrated by the number of chromatogram peaks the complexity of the organics and the infrared spectroscopic studies had pointed out that the major functional groups of the materials were hydroxyl, aldehyde, and carboxylic acids, the use of the GC-IR system made possible the analysis of individual compounds and suggested that the organic micropollutants consisted of homologous compounds.

VI. DISCUSSION

Organic micropollutants recovered from Missouri waters were found to be very complex materials composed of many homologous compounds and to exhibit extreme synergistic long-term toxic effects at concentrations considerably lower than the acute toxicity levels. The long-term toxic concentration of these materials could be reliably estimated from short-term data using a mathematical relationship developed in this investigation. Instrumentation and methodology which can be of material aid in the study of trace organics has been evaluated.

A. ORGANIC MICROPOLLUTANTS USED IN THIS STUDY

The trace organics employed in this investigation were recovered from three subsurface waters (Meramec Spring and two deep wells) and a treated river water (Missouri River at St. Louis) using the carbon adsorption method. Because of their many possible origins, these organics were expected to be very complex materials. The Missouri River receives organic pollution from many sources, including industrial and domestic wastes, agricultural runoff, accidental spillage, and bioresistant metabolic byproducts of natural biota. Organic materials recovered from untreated Missouri River water have been shown to be resistant to biological degradation under both simulated stream (37) and waste treatment (9) conditions, to chemical oxidation (12, 13), and to removal by most physiochemical water treatment methods (11). The majority of these materials would thus be expected to pass through the water treatment processes unaltered in nature and concentration. This is emphasized by the work of Ryckman, et al. (36) who studied the concentration of organic micropollutants in raw and treated Missouri River water at eight water treatment

plants along an 800 mile stretch of the river and found that at some of the plants the concentration of organics in treated water was equal to or greater than their concentration in raw water. The subsurface waters sampled originated in an area of Missouri which is noted for its karstic features; the presence of solution channels and sinkholes in this area could have resulted in direct surface water contamination of these water sources. The quality of the water in one of the two deep wells at the time of sampling points to this possibility; the 1150 foot deep UMR well was condemned because of the high coliform count of the water and is now sealed off. Because of the limited industrialization of this area, the possible sources of the subsurface water organics would include primarily domestic wastes, agricultural runoff and infiltration, and bioresistant metabolic byproducts of the natural soil biota.

Because these organic micropollutants were recovered from natural waters, they were appropriate materials for the determination of the toxic effects of trace organics in water and the evaluation of toxicity and identification procedures. The complexity of the organics presented several difficulties in the evaluation of the procedures which probably would not have been encountered if less complex or known materials had been employed; however, evaluation with simple compounds would have limited the application of these procedures.

B. NATURE AND IDENTITY OF THE ORGANIC MICROPOLLUTANTS

Infrared and gas chromatographic analysis of the trace organics was employed in the identification studies. Infrared spectra of the complex surface and subsurface water organics and corresponding group breakdown fractions exhibited the same major absorption bands and indicated that the majority of these materials

were composed of aliphatic alcohols, aldehydes, and carboxylic acids; the presence of aromatic groups was also indicated in some of the trace organics. The presence of other elements such as nitrogen, phosophorus, and sulfur was not indicated in the infrared spectra although these elements had been found in the trace organics by elemental chemical analysis (see Table 9, p. 41). In most cases, however, these elements were present in very small amounts and may not have been detected by infrared analysis. An exception was the CCE recovered from Unit #1 of the UMR well study which contained a large amount of sulfur (27 per cent). It is possible that sulfur was present in this extract in a chelated form, rather than as part of its molecular composition, and was separated from the CCE during the final evaporation of the chloroform to obtain the organic residue. Evidence of this separation was shown when the CCE was redissolved and a yellow insoluble material resulted. The complexity of the trace organics was demonstrated by the number of chromatogram peaks resulting from gas-liquid chromatographic analysis of these materials. It is recognized that pyrolysis of the sample could have occurred and resulted in a large number of compounds not originally present; on the other hand, all the components of the organics may not have been eluted from the column and even those chromatograms with greater than 20 peaks may not represent all the compounds initially present. Since the complex nature of the extracts and group breakdown fractions made the identification of these materials difficult, collection of the materials corresponding to individual chromatogram peaks (which should be individual compounds) and infrared analysis of these samples were used to facilitate the identification of the trace organics. The collected

chromatogram peaks, group breakdown fractions, and parent organics exhibited similar absorption bands indicating that the trace organics were composed of homologous aliphatic compounds containing alcohol, aldehyde, and carboxylic acid functional groups. Some additional information on the nature of these materials was obtained by general chemical characterization studies.* Titration of a solution of the CCE and the CAE recovered from Meramec Spring Run #1 Unit #1 with sodium hydroxide pointed to the presence of four carboxylic acid groups, while the presence of phenolic compounds in the CCE was indicated by paper chromatographic analysis. The presence of aromatic rings was also evident in the infrared spectra of some of the group breakdown fractions of this extract (see Table 25, p. 127).

Considering the homologous nature of the trace organics, their functional groups, their complex nature and probable origin, these materials could be classified in the broad category of humus or humic acids. The term humic acids has been used by other investigators (8, 32, 76) to describe organic materials recovered from subsurface and surface waters. Robinson, et al. (8) on the basis of infrared analysis and titration studies concluded that organic materials recovered from Illinois ground water were composed of carboxylic acids and could be classified as humic acids. Black and Christman (32) have reported that color causing trace organics in surface water were polyhydroxy, methoxy carboxylic acids which were composed of closely related aromatic compounds connected by

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*Performed in the Biochemistry Laboratories of the Chemistry Department, University of Missouri - Rolla, Rolla, Mo.

aliphatic side chains forming a very complex substance. A homologous nature was also indicated for the organic micropollutants studied in this investigation. Shapiro (76) has found that color causing trace organics in surface water were aliphatic, unsaturated, polyhydroxy dicarboxylic acids. Although the organics studied in the present investigation were aliphatic in nature, they exhibited no evidence of unsaturation.

While the results of the identification studies using gas chromatography and infrared analysis showed that the surface and subsurface water organics had similar functional groups, the results of the group breakdown separations indicated that the trace organics possessed different chemical characteristics. The subsurface water organics recovered by each unit of a run and by the corresponding units of the different runs exhibited considerably different solubility behavior. Most noteworthy was the behavior of the alcohol soluble organics obtained with the first unit of each of the two Meramec Spring and the UMR well runs which were not ether soluble. The solubility characteristics of the surface water CCE were very similar to those reported by Grigoropoulos and Ryckman (13) for a composite CCE recovered from Missouri River water but were very different from the characteristics of the subsurface water CCE materials. The apparent lack of correlation between the functional group similarity of the organics and their different solubility partitioning behavior could be explained in part by considering the complexity of these substances. The organic micropollutants appear to be large, complex aliphatic materials composed of homologous compounds. If the length and branching of the aliphatic chains were of sufficient size to permit coiling or overlapping, the trace organics would exhibit

different chemical characteristics depending on which of their functional groups were exposed or hidden. The synergistic toxic behavior of the subsurface water chloroform and alcohol soluble materials could also be explained in a similar manner; the two extracts could have combined when mixed together and formed a complex substance with different chemical characteristics and behavior.

The use of other sophisticated equipment, such as a mass spectrometer or a nuclear magnetic resonance analyzer, could assist in identifying further the complex organics and their effectiveness in performing this task should be evaluated. It should be emphasized, however, that while the cost of a gas chromatograph or an infrared analyzer may not be out of the reach of some of the larger water and waste water treatment plant laboratories, the cost and special requirements of this equipment would limit its use to research laboratories. The development of characterization and identification techniques which could be employed by water and waste water treatment laboratories was one of the objectives of this study.

C. LONG-TERM TOXICITY OF THE ORGANIC MICROPOLLUTANTS

Although the various extracts were shown to possess similar functional groups, the effect of these materials on fish was found to be very different. The CCE and CAE materials recovered from the subsurface water when studied individually did not exhibit acute toxic effects, while the surface water CCE was toxic at a relatively low concentration. Of greater importance was the strongly synergistic toxic behavior of the spring water CCE and CAE which was evidenced when these materials were combined at their naturally occurring ratio. When the exposure time was lengthened, all the organic micropollutants were toxic to

fish at much lower concentrations; however, the relative relationship between acute and long-term values was quite different for the various extracts. The acute and long-term TLm values determined for the different types of fish used in this investigation are summarized in Table 26 in order to facilitate the evaluation of the overall toxicity of the trace organics.

Because of the length of time involved in performing a long-term bioassay, the amount of material required, and the difficulties encountered in maintaining the test fish in the laboratory for extended periods, the development of a mathematical relationship which could be employed to estimate the long-term toxicity, as well as the toxicity at any given time, from a short-term or acute toxicity study was highly desirable. Ideally, the threshold concentration (the concentration which is just on the borderline of toxicity) would be the eventual toxic level which should be estimated; however, due to the variation in resistance of individual fish even among the same species, this concentration would be almost impossible to determine. On the other hand, it was believed that the median tolerance limit (the concentration which would kill only 50 per cent of the fish) could be reliably estimated. In developing the mathematical model, it became apparent that the size and other characteristics of the fish had a pronounced effect on its resistance to a toxicant. This was clearly shown by the 24 hour TLm values of 201 and 88 mg/l for small (5.0 cm) and large (10.5 cm) trout, respectively, exposed to Meramec Spring Run #2 Unit #2 combined CCE and CAE organics. Therefore, a means of normalizing the effects of the fish characteristics had to be developed and before this could be accomplished the mode of action of the organics had to be established.

Table 26

Acute and Long-Term TLm Values

Test Fish		Test Material	Acute TLm, mg/l				Long-Term TLm	
Type	Length, cm/ Weight, g		24 hr.	48 hr.	96 hr.	120 hr.	mg/l	at days
		Meramec Spring CCE & CAE*						
	10.3/16.3	Run #2 Unit #1	130	125	95	82	33.1	40
Trout	10.5/16.0	Unit #2	88	75	61	56	3.9	70
	10.1/16.1	Unit #3	No effect up to 180				--	--
		Missouri River at St. Louis						
	5.3/ 2.8	CCE	36	32	28	24	0.44	80
		Meramec Spring CCE & CAE*						
Red	5.0/ 2.0	Run #1 Unit #1	No effect up to 240				--	--
Shiners	2.9/ 1.3	Unit #2	No effect up to 200				--	--
	2.8/ 1.2	Run #2 Unit #1	No effect up to 305				--	--
	5.7/ 2.3	Unit #2	195	170	148	120	13.3	50
		Meramec Spring CCE & CAE*						
	7.5/ 9.1	Run #2 Unit #1	166	141	115	103	25.4	50
Sunfish	7.0/ 8.7	Unit #2	137	121	114	100	47.0	50
		Missouri River at St. Louis						
	7.7/11.7	CCE	56	49	45	39	30.1	30
		Meramec Spring CCE & CAE*						
Golden Shiners	6.4/ 4.0	Run #2 Unit #2	180	171	160	152	141	20
		Missouri River at St. Louis						
	6.6/ 4.3	CCE	59	52	39	33	7.5	40

*Combined at naturally occurring ratio.

Visual observations, respiratory enzyme studies using a Warburg respirometer, and oxygen transfer determinations using an especially designed experimental model were employed to evaluate the mode of action of the organic micropollutants. Visual observations of the organs of the fish which had died from exposure to the surface and subsurface water organics indicated that the heart or liver and gills, respectively, were damaged. The fish before death appeared to be suffocating and, consequently, disruption of external or internal oxygen transfer was suspected as the cause of death. The surface water CCE was found to inhibit respiratory enzyme activity, while the subsurface water CCE and CAE materials, individually or combined, did not affect this activity. On the other hand, the surface water CCE and the individual subsurface water CCE and CAE materials did not significantly impair the transfer of oxygen across a semipermeable membrane, but the combined subsurface water organics completely clogged this membrane. This synergistic behavior of the subsurface water CCE and CAE was very similar to the action observed in the acute and long-term bioassay studies. Accumulation of a large amount of material was observed on the gills of test fish exposed to the combined spring extracts and could possibly indicate that another factor was involved in the toxicity of these materials. In addition to clogging the gills, these trace organics could have acted as irritants causing an excessive amount of mucous material to be secreted and accumulated on the gills, thus reducing the flow of oxygen across the gills. A similar phenomenon has been reported by Westfall (77) for fish exposed to lead nitrate. Based on the findings of these studies, it was concluded that the mode of action of the surface and subsurface water extracts was

an inhibition of respiratory enzyme activity and a physical blockage of oxygen transfer across the gills, respectively. The application of the procedures employed in the mode of action studies is not limited to these materials, but could be used with other substances in similar investigations. The respiratory enzyme and oxygen transfer procedures provide a rapid (less than 120 minutes test time) means of indicating whether a substance will be toxic to a living system and the general way in which it will affect the system.

In order to normalize the effect of the mode of action of the toxicant and of the physical characteristics of the fish on the TLm concentration, the toxicity factor concept was developed. In defining the toxicity factor, it was considered highly desirable to keep the data required for this relationship as simple as possible without, however, impairing the accuracy of the mathematical model. Since the mode of action of the subsurface water organics was a physical blockage of oxygen transfer across the gills, the toxicity factor (y_t) for these materials was defined as a relationship between TLm value (C_t) and fish characteristics (gill surface area, GSA, and condition factor, K) which was expressed as follows:

$$y_t = C_t \cdot \left[\frac{GSA}{K}\right]^{1.25}$$

The condition factor was employed to represent the demand for oxygen by the fish which controlled the amount of oxygen transported across the gills. Since the gills were not physically affected by the surface water extracts, the gill

surface area was not included in the toxicity factor relationship for these materials. A toxicity factor expressed as

$$y_t = C_t/K$$

was found to relate successfully fish characteristics and the corresponding TLm value. The effect of the surface water CCE on the respiratory enzymes in liver, heart, and gill tissue homogenates is not directly reflected in this relationship because its exact nature has not been determined. The same relationship was used to define the toxicity factor for the pesticide malathion which was employed to provide data for a known compound. Visual observations and tissue studies indicated that the mode of action of this substance was different from that of either the surface or subsurface water organics. Although the mode of action of malathion was not evaluated further, it has been reported in the literature (68, p. 742) that this chemical inhibits brain cholinesterase activity.

An empirical toxicity equation relating the toxicity factor (y_t) with the exposure time (t) was developed from experimental data and had the general form:

$$y_t = y_c + (y_o - y_c)\, e^{-bt}$$

The terms y_o and y_c represent the immediate and eventual toxicity factors while b is a constant. The value of y_c, y_o, and b for a given fish and toxicant can be obtained from an acute bioassay and a general knowledge of the mode of action. The term y_c will normally be very small compared to y_o but cannot be omitted from the equation because it is a measure of the eventual or long-term TLm

value. It should be again emphasized that y_t is not the TLm concentration but rather it is the relationship between this concentration and fish characteristics at a given time.

The mathematical model was evaluated with long-term toxicity values determined in this investigation or reported in the literature (69) and was found to provide satisfactory results. The theoretical (estimated) and experimental values were very close in almost all cases as evidenced by the fact that the theoretical value in the majority of these cases deviated from the experimental value by less than 10 per cent ranging from a low of 2 per cent to a high of 40 per cent. The mathematical model may not permit the prediction of exact long-term levels, but it does enable a close estimation of the general range in which the TLm value should fall. Additional work is necessary to evaluate the applicability of the model to a wider range of toxicants and extend its usefulness to other test animals.

Although the acute TLm values determined in this investigation may not be reached under natural conditions, some of the long-term levels could be approached; these concentrations would have to persist for a long period of time before the health of aquatic life is endangered if the organics were acting alone. Synergistic action between the various trace organics and other materials in the water could significantly magnify the effect of the organic micropollutants.

D. INSTRUMENTATION AND METHODOLOGY FOR THE STUDY OF THE ORGANIC MICROPOLLUTANTS

The instrumentation utilized in the identification phase of this study was employed for two reasons. The presently used characterization techniques

reveal very little of the identity of the trace organics and, consequently, more capable instrumentation and specific procedures are needed to provide a greater insight of the nature of these materials. Also, many water treatment plant laboratories are searching for a better means of detecting and characterizing the organic micropollutants present in water. Gas chromatography and infrared spectroscopy could provide the research tools required for the identification of the trace organics and function as practical control instruments which could be used in water treatment plant laboratories.

Gas chromatography permitted the detection of minute quantities of organic materials (much less than 1 mg/l), but direct aqueous injection as advocated by Baker (53) for the detection of trace organics would not be possible with most waters because of the concentration at which these materials are normally found. Although most of the trace organics could be separated by gas chromatography, difficulty was encountered in separating some of these materials; this in some cases could limit the application of this technique. Caruso, et al. (26) have recently proposed the use of gas chromatographic analysis for the detection of organic micropollutants in water and have suggested that the resulting chromatograms could function as "fingerprints" or characteristic separation patterns of particular organic materials and could be employed to indicate the presence of troublesome contaminants. The type and degree of water treatment could be adjusted on the basis of these fingerprints to provide the desired quality of the finished water. The use of the technique suggested by Caruso and his coworkers is presently being evaluated at several water treatment plants along the Missouri River (61); however, complete

evaluation of its applicability has been hampered by the need for a concentrating procedure prior to gas chromatographic analysis. The requirement of a concentrating technique and the inherent limitations of gas chromatography may reduce the effectiveness of the fingerprinting procedure. However, if an acceptable concentrating method is developed, the application of gas chromatographic separation coupled with infrared analysis of collected chromatogram peaks might provide the water treatment laboratories with a better monitoring technique than the fingerprinting method now being evaluated. The additional information provided by the infrared spectra could be employed to characterize further the trace organics. Although the collection system employed in the identification studies of this investigation performed satisfactorily with organic micropollutants recovered from surface and subsurface water, the final evaluation of this technique must necessarily be made at the individual treatment plant. The infrared spectra of the individual chromatogram peaks would represent the absorption bands for one compound and, therefore, considerably simplify the characterization of these materials. Direct infrared analysis would also be of material aid to both water plant and research personnel in identifying the trace organics. This would be especially true for those organic substances which cannot be separated by the gas chromatograph and for which infrared analysis of collected peaks cannot be performed.

The Beckman carbonaceous analyzer employed to determine the carbon content of the various organic micropollutants gave values which when expressed as a percentage of weight of the trace organic material were very close to the values determined as part of elemental chemical analysis of selected

extracts. The time required for the carbon analysis of a sample using this instrument is very short (about 2 minutes); however, warm-up and standardization usually required from 1 to 2 hours. Although a carbon determination requires a small volume of sample (usually 20 μl), the minimum detectable carbon concentration is in the mg/l range and, consequently, carbon determinations for the organic materials by direct injection is not possible. However, as in the case of gas-liquid chromatography, if a rapid concentrating technique is developed, carbon determinations with the carbonaceous analyzer would provide a rapid, accurate control parameter of water quality.

Acute and long-term toxicity studies were performed using a batch-type bioassay procedure which permitted the use of a minimum amount of trace organics and provided a simple and rapid technique for evaluating the acute toxicity of the organics. It is recognized that this method has several potential limitations, including the possibility of toxicant concentration changes with time and loss of volatile materials by aeration, and the small number of test fish utilized; however, a better procedure has not yet been developed. An additional limitation of the batch-type long-term bioassay is the prolonged time during which the fish are held under static conditions; this may cause a higher frequency of disease in the fish than what would have occurred under continuous flow conditions. The test volume normally specified (16, p. 551) for a batch-type bioassay (one liter of solution for each 2 grams of fish weight) would necessitate the use of a large quantity of organic micropollutants which may not always be available. Satisfactory bioassays, both acute and long-term, were performed in this investigation with a relatively small volume of test solution. The

mathematical model developed in this study significantly extends the usefulness of the acute bioassay. Data made available by this standard test coupled with an understanding of the mode of action of the toxicant obtained by means of the simple and rapid respiratory enzyme and oxygen transfer studies provide all the information which is required for the accurate prediction of the long-term effect of the toxicant using the mathematical model.

The carbon adsorption method provided an adequate means of recovering organic micropollutants from both surface and subsurface water. It enabled the sampling of a large volume of water and the resulting recovery of a workable quantity of trace organics. The variety of elutants which were used increased the recovery efficiency, both qualitatively and quantitatively. Modification of the method to employ large capacity filters permitted the sampling in a two week period of approximately the same volume of water that would have required a year with the PHS size filter. Several possible limitations of the technique have been recognized and include the selective adsorption of the organic materials on the carbon and selective or incomplete desorption with the elutants used; in addition, some of the organic materials may be altered either biologically or chemically while adsorbed on the carbon. These limitations and the desire for a more rapid recovery procedure have encouraged other investigators to evaluate liquid-liquid extraction and freeze-drying as recovery procedures. Although these methods are more rapid and reportedly more efficient than carbon absorption, they are capable of sampling only a limited volume of water and require the use of a solvent which is immiscible with water. Inorganic materials dissolved in the water seriously affect the freeze-drying technique. The

carbon adsorption method was, therefore, employed in this investigation with full recognition of its shortcomings because it was considered to be the best procedure available for recovering sufficient quantities of organics to permit extensive identification and toxicity studies. If the carbon adsorption method could be modified to reduce the time required for sampling, drying, and elution, it would permit a more rapid sampling procedure than is now employed. While the time for recovery would not be so fast as that advocated for the other recovery methods, the carbon adsorption method would possess the distinct advantage of the choice of solvents which can be used and the quantity of material which can be obtained. Aspects of the standard carbon adsorption procedure which could be modified include a sizeable increase in the filtration rate, a significant reduction in the filtration time, the elution of partially dried carbon, and a decrease in the elution time. These modifications would undoubtedly reduce the efficiency of the method, however, a measurable representative quantity of the trace organics could still be recovered in a much shorter period of time. Another possible modification would involve the desorption of the organics without the use of a Soxhlet extraction apparatus in a procedure analogous to liquid-liquid extraction.

E. ORGANIC MICROPOLLUTANTS IN WATER

Although many studies have been performed in the field of organic micropollutants and have contributed significantly to the existing knowledge in this area, a considerable amount of work remains to be done. The mode of action of the toxic organics should definitely be evaluated further, especially with regard to the synergistic or antagonistic effects. The complete identification

of the organic micropollutants is a prerequisite to the full evaluation of the aesthetic and particularly the toxic effects of the trace organics and the development of methods for their removal. The primary responsibility for identifying the trace organics lies with research laboratories; however, the necessary procedures should be developed so that they could be employed at water or waste water treatment plants to aid in more efficient and effective plant operation.

The recovery of significant quantities of organic materials other than those which are chloroform soluble and the extreme synergistic effects demonstrated in this study strongly point to the necessity for reevaluating the present drinking water standards with respect to both the quantity and type of organics permissible in the water. The 200 μg/l limit for chloroform soluble organics was established on the basis of the known aesthetic potential and unknown toxicological characteristics of these materials. The results of this investigation emphasize the inadequacy of this limit and the need for its reexamination in the light of the new findings.

VII. CONCLUSIONS

On the basis of the findings of this investigation, the following conclusions can be drawn:

1. The modified carbon adsorption method using three large capacity (1.5 cubic foot) units in series and sequential elution with a series of solvents was effective in recovering sufficient quantities of organic micropollutants from subsurface water for extensive characterization, identification, and toxicity studies. Significant concentrations of trace organics were found in Meramec Spring water and detectable concentrations were present in the UMR and Rolla deep well waters.

2. The organic micropollutants recovered from these subsurface water sources and treated Missouri River water were most appropriate materials for use in this study and best represented the trace organics found in water under natural conditions.

3. Gas-liquid chromatography was capable of separating the majority of the organic micropollutants, but could not separate the CAE materials. Collection of individual chromatogram peaks for postchromatographic infrared analysis was possible. The use of gas-liquid chromatographic analysis demonstrated the complex nature of the trace organics but could not alone attain the identification of these materials.

4. Partial identification of the surface and subsurface water organic micropollutants was possible by an evaluation of spectra obtained by direct infrared analysis and infrared spectra of collected gas chromatograph peaks.

5. The surface and subsurface water organic micropollutants were composed primarily of homologous compounds containing aliphatic alcohol, aldehyde, and carboxylic acid functional groups; aromatic groups were also detected in some of the trace organics. On the basis of their functional groups, chemical characteristics, and probable origin, the trace organics can be placed in the broad category of humic acids.

6. The chloroform and alcohol soluble organic micropollutants recovered at Meramec Spring were not individually toxic to fish but in many cases exhibited strong synergistic behavior when combined at their naturally occurring ratio.

7. The treated Missouri River water CCE and many of the Meramec Spring water combined CCE and CAE materials were toxic to fish at both high concentrations over a short exposure time and at lower concentrations over an extended time. The surface water CCE was considerably more toxic than the most toxic combined subsurface water trace organics; the 24 hour TLm values for trout exposed to these materials were 36 and 201 mg/l, and the corresponding long-term TLm values were 0.44 mg/l in 80 days and 10 mg/l in 70 days.

8. Respiratory enzyme studies using trout tissue homogenates in a Warburg respirometer and oxygen transfer studies using a semipermeable membrane in an especially designed test unit were effective in defining the mode of action of the organic micropollutants.

9. The mode of action of the surface and subsurface water organic micropollutants was a disruption of respiratory enzyme activity and a physical blockage of oxygen transfer across the gills, respectively.

10. The toxic concentration of the organic micropollutants depended upon the mode of action of the toxicant and the physical characteristics of the test fish. The toxicity factor concept developed in this study adequately normalized the effect of these variables. The toxicity factor (y_t) for the surface and subsurface water trace organics can be defined as

$$\text{Surface: } y_t = C_t/K \qquad \text{Subsurface: } y_t = C_t \cdot \left[\frac{GSA}{K}\right]^{1.25}$$

where C_t, GSA, and K are the TLm concentration, gill surface area, and condition factor of the fish, respectively.

11. The long-term median tolerance limit can be accurately estimated by means of the toxicity equation developed in this study. This equation can be defined as

$$y_t = y_c + (y_o - y_c)\, e^{-bt}$$

where y_o and y_c are the immediate and eventual toxicity factors, y_t is the toxicity factor at any time t, and b is a constant.

12. The procedures which were evaluated for the characterization, identification, and evaluation of the long-term toxic effects of the organic micropollutants can be employed equally well in treatment plant laboratories to monitor treated water and waste effluent quality and in research laboratories

to investigate further the trace organics. To meet the challenge of organic micropollutants in water, practical application of these procedures at the larger treatment plants is very essential.

13. The 200 μg/l limit for chloroform soluble organics set by the Public Health Service drinking water standards needs to be reevaluated. Trace organics other than the chloroform soluble materials are found in water at significant concentrations and possess strong synergistic toxic behavior.

VIII. RECOMMENDATIONS FOR FUTURE RESEARCH

During the course of this investigation, the following areas meriting further study were exposed:

1. The carbon adsorption method should be modified to enable the recovery of workable quantities of organic micropollutants in a short period of time and increase the usefulness of this technique as a monitoring agent.

2. The mode of action of the toxic organic micropollutants should be evaluated further with emphasis on the cause of their synergistic or antagonistic behavior.

3. The applicability of the long-term mathematical model to a wider range of toxicants and test animals should be evaluated.

4. The identity of the organic micropollutants should be determined as completely as possible to permit the full evaluation of their aesthetic and particularly their toxic effects and the development of methods for their removal. Consideration should be given to the adaptation of the identification techniques for use in water and waste water treatment plant laboratories.

5. Effective methods for the destruction and/or removal of organic micropollutants from water should be developed and their applicability to the water and waste water treatment field should be evaluated. Knowledge of the identity of these materials would be of material aid in this important task.

6. The necessary investigations to provide sufficient and appropriate data for the reevaluation of the maximum permissible limit of organic micropollutants in water should be undertaken. These would include the areas of an appropriate recovery system, quantities and types of trace organics present in water, and the detrimental effects of these materials.

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APPENDICES

APPENDIX A

RECOVERY OF ORGANIC MICROPOLLUTANTS FROM MISSOURI SUBSURFACE WATER

Two modified carbon adsorption assemblies, each consisting of three filters in series, were employed to recover trace organics. Each unit contained a 0.75 cubic foot central layer of fine carbon* held between two 0.38 cubic foot end layers of coarse carbon.** The dimensions and construction details of a typical filter are shown in Figure A-1, and the filter arrangement employed at each location is presented in Figure A-2. At Meramec Spring, water was supplied to the filters with a 1.5 horsepower centrifugal pump, while at each well the existing deep well impeller-type pump was utilized with the excess water being wasted at the UMR well and pumped to the City's distribution system at the Rolla well. The filter arrangement at Meramec Spring and the UMR well are shown in Figure A-3.

After the desired quantity of water had been filtered, the carbon was removed from the filters and brought to the laboratory; it was then placed in wooden trays lined with polyethylene and dried at 40°C for 5 days. The organic matter adsorbed onto the carbon was sequentially extracted with redistilled chloroform, ethanol, and then acetone and benzene, or benzene and acetone using four modified Soxhlet extractors.*** Chloroform was chosen

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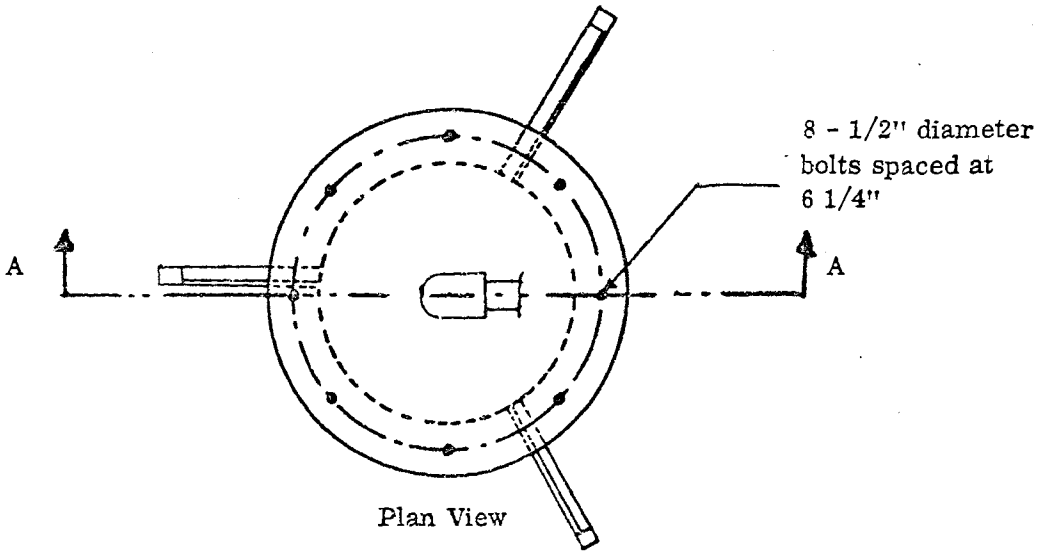
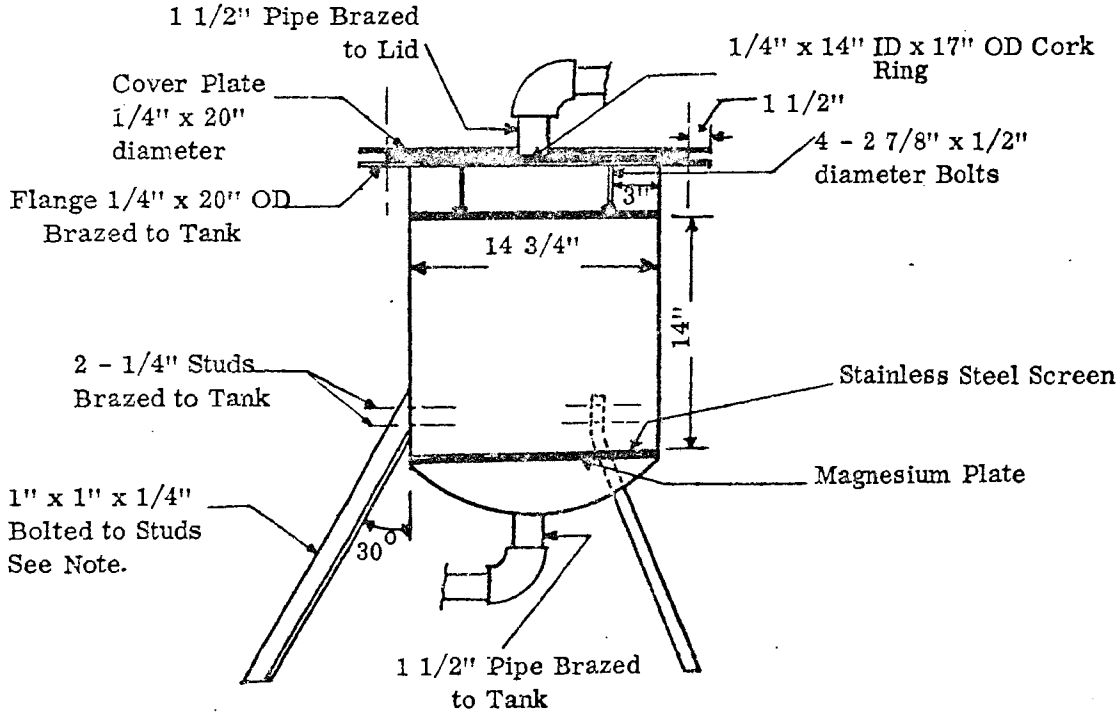
*Nuchar C-190, +30 mesh, a product of the West Virginia Pulp and Paper Company, New York, N.Y.

**Cliffchar, 4 x 10 mesh, a product of the Cliffs Dow Chemical Company, Marquette, Mich.

***Pyrex No. 3885, a product of Corning Glass Works, Corning, N.Y.





Section A-A

Scale 0.01" = 1.0"

Note:
All brazed joints to be pressure tight; three legs spaced at 15 1/2".

Figure A-1

Activated Carbon Filter

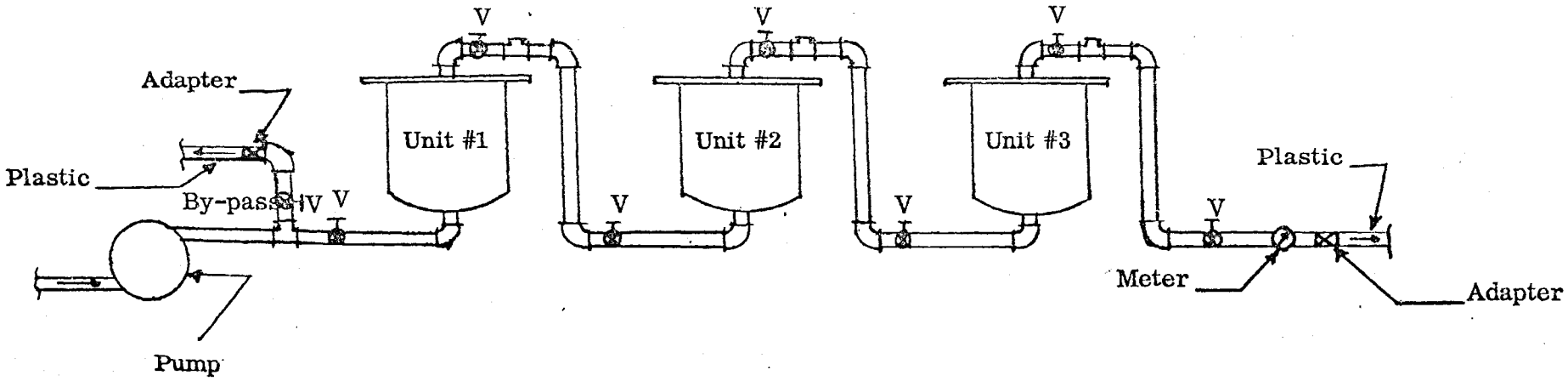


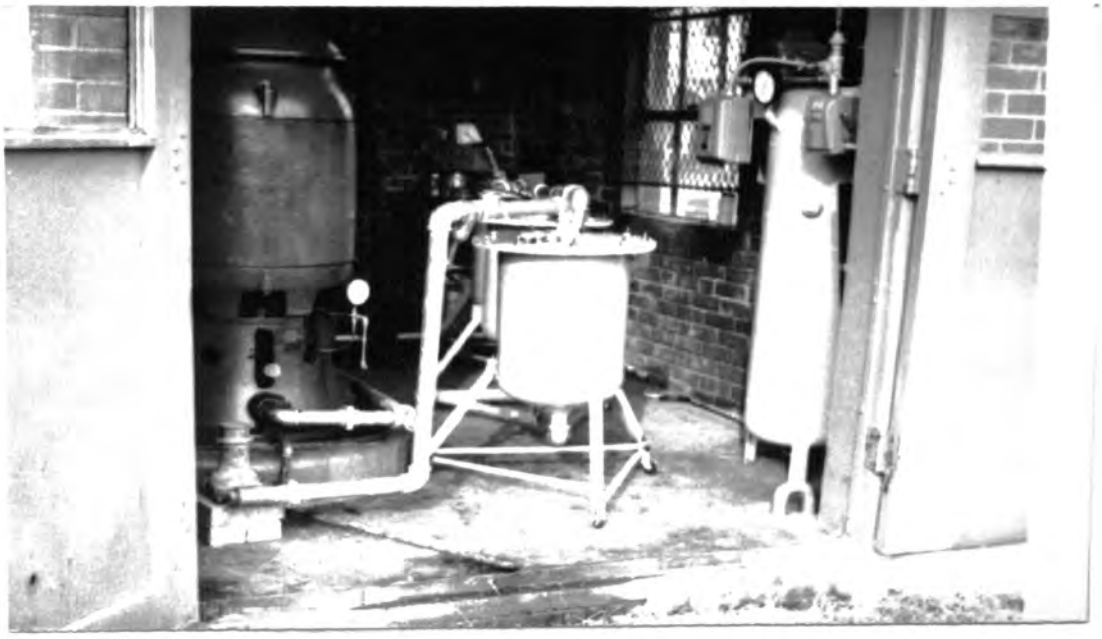
Figure A-2

Activated Carbon Filter Arrangement

Scale 0.01" = 1.0"

Note: All pipes 1 1/2" galvanized iron except as noted.

Meramec Spring



UMR Well

Figure A-3

Activated Carbon Filter Arrangement
For Sampling Subsurface Waters

because it has been shown (15) that the odor causing materials in surface waters are chloroform soluble. Ethanol, acetone, and benzene were used in an attempt to recover other materials present. Other investigators (9,20) have reported that these elutants did recover significant quantities of organics from surface waters. The extraction procedure used was basically as outlined in Standard Methods (16, p. 215) with the exception that the extraction time was reduced from 36 to 24 hours because of the large volume of carbon which was to be extracted. After a volume of carbon had been extracted for 24 hours (48 cycles), the excess solvent was distilled off leaving about 200 ml which contained the organics. This remaining volume was filtered through solvent rinsed filter paper* and sintered glass to remove any carbon fines carried into the solvent during the extraction process. The solvent containing the organic materials was then evaporated off using a water bath and a gentle jet of carbon-filtered air directed on the surface of the liquid. The organics from the different extractions which used carbon from the same filter were then combined to give the total quantity of organic micropollutants recovered from a filter.

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*Whatman #40, a product of the H. Reeve Angel and Company, Inc., Clifton, N. J.

APPENDIX B

RESPIRATORY ENZYME STUDIES

These studies were performed to evaluate the toxicity of the various test materials on respiratory enzyme systems and to assist in determining the mode of action of the trace organics. A Warburg respirometer equipped with 15 ml reaction vessels was employed to measure the effect of the presence of the organic micropollutants and pesticides on the oxygen utilization by respiratory enzyme systems in trout tissue homogenates.

Tissue stock solutions containing 67, 33, and 20 mg/ml of liver, heart, and gill tissue were employed. To prepare the stock solutions, three healthy medium to large size trout were sacrificed, and their organs were removed and weighed; 13.4, 6.6, and 4.0 g portions of liver, heart, and gills, respectively, were homogenized in a Waring blender at 5°C, mixed with enough isotonic solution to provide a final volume of 200 ml, and stored at 5°C. The average weight of the liver, heart, and gills of the 12 to 16 inch trout was 0.79, 0.42, and 0.30 per cent, respectively, of the total fish weight (570 grams average); therefore, the organs from three fish provided adequate quantities of tissues. It is interesting to note that the weight of the heart was a relatively constant amount of the total body weight, while the weight of the liver and gills was not.

The oxygen uptake of the tissue homogenates, in mg/l, was calculated from the change in the manometer reading, in mm, utilizing a flask constant computed by the procedure given in Manometric Techniques by Umbreit, et al. (65, p. 61). The oxygen uptake was related to the quantity of tissue present

in the flask by dividing the uptake (mg/l) by the tissue concentration (g/l) in the reaction vessel and was expressed as mg oxygen per g tissue.

Several different compounds were evaluated as substrates for the enzume studies using trout liver homogenate and the corresponding oxygen uptake values are presented in Table B-1. Succinic acid was selected as a substrate because of the rapid initial uptake and the short time required for respiration to level off. The desirable substrate concentration was determined by exposing liver homogenate to various concentrations of succinic acid, and the results are also shown in Table B-1. The oxygen uptake values given in Tables B-1 have been previously presented graphically in Figure 4, p. 61.

Oxygen uptake values for trout tissue homogenates in the presence of subsurface and surface water extracts and pesticides are presented in Tables B-2, B-3, and B-4, respectively. The subsurface water organics and pesticides exerted no effect on the enzyme activity, while the surface water CCE inhibited this activity.

The 60 minute oxygen uptake values, expressed as a percentage of the control, for the various concentrations of surface water CCE materials are summarized in Table B-5. These values were used to determine the 60 minute median respiratory tolerance limit for the trout homogenates as shown in Figure 7, p. 66.

Table B-1

Oxygen Uptake by Trout Liver Tissue Homogenate
Exposed to Succinic Acid and Other Substrates

Time Minutes	Oxygen Uptake, mg oxygen/g tissue*							
	Sucrose 0.1M	Glucose 0.1M	Citric Acid 0.1M	Succinic Acid				
				0.1M	0.01M	0.05M	0.15M	0.2M
5	0.04	0.04	0.07	0.10	0.02	0.06	0.11	0.12
10	0.07	0.08	0.11	0.13	0.03	0.09	0.14	0.15
20	0.09	0.10	0.13	0.17	0.04	0.12	0.18	0.20
30	0.14	0.14	0.17	0.23	0.05	0.14	0.24	0.25
45	0.19	0.18	0.22	0.28	0.08	0.17	0.30	0.32
60	0.22	0.20	0.25	0.29	0.13	0.21	0.33	0.36
90	0.24	0.24	0.28	0.35	0.13	0.27	0.41	0.45
120	0.27	0.28	0.30	0.36	0.13	0.27	0.46	0.49

*Tissue concentration: 22.7 g/l.

Table B-2

Oxygen Uptake by Trout Tissue Homogenates
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #1 Unit #1*

Time Minutes	Oxygen Uptake, mg oxygen/g tissue														
	Liver, 22.7 g/l					Heart, 11.0 g/l					Gill, 6.7 g/l				
	Concentration, mg organics/mg tissue														
	0.0	0.09	0.27	0.54	1.08	0.0	0.18	0.54	1.08	2.16	0.0	0.30	0.90	1.80	3.60
5	0.10	0.11	0.10	0.10	0.11	0.12	0.12	0.11	0.10	0.11	0.04	0.04	0.04	0.04	0.04
10	0.15	0.14	0.14	0.13	0.16	0.18	0.17	0.16	0.15	0.16	0.10	0.09	0.10	0.08	0.10
20	0.19	0.16	0.15	0.14	0.19	0.20	0.18	0.18	0.17	0.20	0.14	0.13	0.13	0.11	0.15
30	0.24	0.19	0.18	0.19	0.24	0.22	0.20	0.19	0.18	0.22	0.16	0.14	0.14	0.12	0.17
45	0.26	0.21	0.21	0.21	0.25	0.26	0.23	0.22	0.22	0.25	0.22	0.18	0.19	0.18	0.23
60	0.28	0.24	0.23	0.22	0.26	0.30	0.26	0.25	0.24	0.27	0.24	0.21	0.22	0.20	0.24
90	0.29	0.25	0.25	0.24	0.28	0.31	0.27	0.27	0.25	0.29	0.28	0.23	0.24	0.22	0.26
120	0.30	0.27	0.28	0.26	0.29	0.32	0.28	0.28	0.27	0.30	0.28	0.24	0.25	0.23	0.28

*CCE and CAE combined at naturally occurring ratio of 1 to 1.56.

Conditions:

1. Each Warburg flask contained:
   1 ml 0.3M succinic acid
   1 ml tissue homogenate
   1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-2 (Continued)

Oxygen Uptake by Trout Tissue Homogenates
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #1 Unit #2*

Time Minutes	Oxygen Uptake, mg oxygen/g tissue														
	Liver, 22.7 g/l					Heart, 11.0 g/l					Gill, 6.7 g/l				
	Concentration, mg organics/mg tissue														
	0.0	0.09	0.27	0.54	1.08	0.0	0.18	0.54	1.08	2.16	0.0	0.30	0.90	1.80	3.60
5	0.15	0.16	0.15	0.15	0.15	0.11	0.11	0.10	0.11	0.10	0.04	0.05	0.04	0.05	0.04
10	0.20	0.20	0.18	0.19	0.20	0.14	0.13	0.14	0.15	0.14	0.08	0.09	0.08	0.10	0.09
20	0.24	0.23	0.22	0.21	0.21	0.19	0.18	0.19	0.19	0.18	0.14	0.13	0.12	0.11	0.12
30	0.28	0.28	0.25	0.27	0.25	0.20	0.20	0.21	0.22	0.23	0.15	0.14	0.15	0.14	0.13
45	0.30	0.31	0.27	0.28	0.29	0.24	0.23	0.25	0.24	0.24	0.20	0.19	0.18	0.17	0.16
60	0.32	0.32	0.30	0.30	0.31	0.26	0.26	0.26	0.25	0.25	0.22	0.20	0.19	0.18	0.19
90	0.35	0.34	0.32	0.33	0.34	0.28	0.27	0.27	0.26	0.27	0.24	0.22	0.21	0.20	0.22
120	0.35	0.34	0.33	0.34	0.34	0.28	0.28	0.27	0.27	0.27	0.25	0.23	0.22	0.21	0.23

*CCE and CAE combined at a naturally occurring ratio of 1 to 3.65.

Conditions:
1. Each Warburg flask contained:
   1 ml 0.3M succinic acid
   1 ml tissue homogenate
   1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-2 (Continued)

Oxygen Uptake by Trout Tissue Homogenates
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #2 Unit #1*

Time Minutes	Oxygen Uptake, mg oxygen/g tissue														
	Liver, 22.7 g/l					Heart, 11.0 g/l					Gill, 6.7 g/l				
	Concentration, mg organics/mg tissue														
	0.0	0.09	0.27	0.54	1.08	0.0	0.18	0.54	1.08	2.16	0.0	0.30	0.90	1.80	3.60
5	0.10	0.11	0.10	0.12	0.11	0.08	0.08	0.09	0.05	0.07	0.04	0.04	0.03	0.05	0.04
10	0.14	0.13	0.14	0.14	0.15	0.10	0.09	0.10	0.06	0.09	0.06	0.05	0.05	0.06	0.06
20	0.18	0.17	0.18	0.19	0.19	0.14	0.13	0.12	0.18	0.13	0.10	0.08	0.07	0.08	0.08
30	0.22	0.20	0.21	0.23	0.23	0.16	0.17	0.15	0.10	0.16	0.12	0.11	0.10	0.10	0.11
45	0.26	0.25	0.25	0.27	0.27	0.22	0.20	0.17	0.13	0.20	0.14	0.13	0.13	0.13	0.14
60	0.28	0.28	0.30	0.31	0.31	0.24	0.23	0.20	0.16	0.23	0.17	0.15	0.16	0.16	0.17
90	0.34	0.33	0.33	0.33	0.33	0.25	0.24	0.22	0.17	0.25	0.19	0.16	0.18	0.17	0.19
120	0.36	0.35	0.34	0.34	0.35	0.27	0.25	0.24	0.18	0.26	0.20	0.18	0.19	0.18	0.20

*CCE and CAE combined at naturally occurring ratio of 1 to 1.48.

Conditions:

1. Each Warburg flask contained:
   - 1 ml 0.3M succinic acid
   - 1 ml tissue homogenate
   - 1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-2 (Continued)

Oxygen Uptake by Trout Tissue Homogenates
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #2 Unit #2*

Time Minutes	Oxygen Uptake, mg oxygen/g tissue														
	Liver, 22.7 g/l					Heart, 11.0 g/l					Gill, 6.7 g/l				
	Concentration, mg organics/mg tissue														
	0.0	0.09	0.27	0.54	1.08	0.0	0.18	0.54	1.08	2.16	0.0	0.30	0.90	1.80	3.60
5	0.18	0.17	0.17	0.18	0.16	0.10	0.09	0.11	0.10	0.09	0.03	0.04	0.04	0.03	0.04
10	0.23	0.21	0.22	0.22	0.21	0.12	0.11	0.12	0.11	0.12	0.08	0.07	0.08	0.09	0.08
20	0.26	0.25	0.26	0.25	0.24	0.17	0.16	0.17	0.16	0.17	0.13	0.12	0.11	0.12	0.11
30	0.30	0.29	0.28	0.29	0.27	0.20	0.20	0.19	0.18	0.19	0.16	0.17	0.15	0.16	0.15
45	0.33	0.33	0.31	0.31	0.30	0.23	0.21	0.22	0.23	0.23	0.18	0.19	0.19	0.19	0.18
60	0.35	0.34	0.33	0.34	0.32	0.28	0.27	0.27	0.27	0.28	0.22	0.20	0.24	0.23	0.21
90	0.37	0.36	0.36	0.36	0.35	0.30	0.32	0.32	0.32	0.32	0.26	0.25	0.25	0.26	0.24
120	0.38	0.38	0.37	0.37	0.36	0.34	0.33	0.33	0.34	0.32	0.28	0.27	0.26	0.28	0.27

*CCE and CAE combined at naturally occurring ratio of 1 to 3.35.

Conditions:

1. Each Warburg flask contained:
   - 1 ml 0.3M succinic acid
   - 1 ml tissue homogenate
   - 1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-2 (Continued)

Oxygen Uptake by Trout Tissue Homogenates
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #2 Unit #3*

Time Minutes	Oxygen Uptake, mg oxygen/g tissue														
	Liver, 22.7 g/l					Heart, 11.0 g/l					Gill, 6.7 g/l				
	Concentration, mg organics/mg tissue														
	0.0	0.09	0.27	0.54	1.08	0.0	0.18	0.54	1.08	2.16	0.0	0.30	0.90	1.80	3.60
5	0.18	0.17	0.19	0.16	0.18	0.10	0.09	0.11	0.10	0.08	0.06	0.04	0.05	0.05	0.04
10	0.24	0.23	0.22	0.19	0.23	0.13	0.11	0.13	0.12	0.13	0.10	0.08	0.08	0.07	0.09
20	0.29	0.30	0.24	0.24	0.25	0.17	0.15	0.17	0.16	0.16	0.12	0.11	0.12	0.10	0.11
30	0.30	0.31	0.26	0.26	0.27	0.20	0.19	0.20	0.19	0.21	0.16	0.14	0.15	0.13	0.14
45	0.31	0.32	0.28	0.30	0.29	0.23	0.22	0.22	0.22	0.24	0.18	0.17	0.17	0.16	0.18
60	0.34	0.33	0.30	0.34	0.32	0.26	0.27	0.26	0.26	0.28	0.22	0.20	0.20	0.20	0.21
90	0.35	0.34	0.35	0.36	0.34	0.30	0.31	0.30	0.30	0.31	0.24	0.22	0.21	0.22	0.22
120	0.38	0.37	0.36	0.38	0.36	0.33	0.32	0.31	0.32	0.33	0.25	0.24	0.23	0.24	0.24

*CCE and CAE combined at naturally occurring ratio of 1 to 2.46.

Conditions:

1. Each Warburg flask contained:
   - 1 ml 0.3M succinic acid
   - 1 ml tissue homogenate
   - 1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-3

Oxygen Uptake for Trout Tissue Homogenates Exposed to Surface Water CCE

Treated Missouri River Water

Time Minutes	Oxygen Uptake, mg oxygen/g tissue																							
	Liver, 22.7 g/l								Heart, 11.0 g/l								Gill, 6.7 g/l							
	Concentration, mg organics/mg tissue																							
	0.0	0.015	0.03	0.045	0.06	0.09	0.24	0.365	0.0	0.03	0.06	0.09	0.12	0.18	0.48	0.73	0.0	0.05	0.10	0.15	0.20	0.30	0.80	1.35
5	0.20	0.20	0.19	0.15	0.10	0.05	0.01	0.00	0.10	0.10	0.10	0.09	0.09	0.08	0.01	0.00	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.00
10	0.25	0.25	0.24	0.18	0.12	0.08	0.02	0.01	0.14	0.14	0.14	0.13	0.13	0.10	0.01	0.00	0.10	0.10	0.10	0.09	0.08	0.08	0.01	0.00
20	0.29	0.29	0.28	0.25	0.18	0.12	0.03	0.01	0.19	0.18	0.18	0.18	0.16	0.13	0.02	0.01	0.15	0.15	0.15	0.14	0.12	0.10	0.03	0.00
30	0.32	0.31	0.31	0.29	0.22	0.15	0.04	0.02	0.22	0.22	0.22	0.20	0.18	0.15	0.03	0.01	0.18	0.17	0.16	0.15	0.13	0.11	0.03	0.00
45	0.33	0.33	0.32	0.30	0.24	0.18	0.06	0.02	0.25	0.25	0.24	0.23	0.22	0.17	0.05	0.01	0.20	0.20	0.19	0.18	0.17	0.12	0.06	0.01
60	0.35	0.35	0.34	0.32	0.26	0.19	0.08	0.02	0.30	0.30	0.29	0.28	0.24	0.17	0.06	0.02	0.24	0.23	0.23	0.23	0.20	0.13	0.08	0.01
90	0.37	0.37	0.35	0.33	0.28	0.21	0.09	0.03	0.32	0.34	0.32	0.30	0.27	0.18	0.09	0.02	0.26	0.26	0.25	0.23	0.22	0.16	0.09	0.01
120	0.39	0.38	0.37	0.34	0.28	0.22	0.10	0.04	0.35	0.35	0.34	0.32	0.27	0.20	0.10	0.03	0.27	0.27	0.26	0.24	0.23	0.17	0.10	0.02

Conditions:

1. Each Warburg flask contained:
   - 1 ml 0.3M succinic acid
   - 1 ml tissue homogenate
   - 1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-4

Oxygen Uptake by Trout Tissue Homogenates
Exposed to Pesticides

Sevin*

Time Minutes	Oxygen Uptake, mg oxygen/g tissue														
	Liver, 22.7 g/l					Heart, 11.0 g/l					Gill, 6.7 g/l				
	Concentration, mg pesticide/mg tissue														
	0.0	0.01	0.27	0.54	2.17	0.0	0.02	0.54	1.08	4.33	0.0	0.03	0.90	1.80	7.20
5	0.18	0.17	0.16	0.17	0.16	0.10	0.09	0.11	0.10	0.11	0.06	0.05	0.04	0.05	0.04
10	0.24	0.23	0.22	0.23	0.22	0.13	0.12	0.12	0.13	0.14	0.10	0.09	0.08	0.08	0.09
20	0.29	0.27	0.26	0.25	0.24	0.17	0.16	0.15	0.16	0.16	0.12	0.11	0.12	0.11	0.12
30	0.30	0.29	0.29	0.28	0.27	0.20	0.19	0.18	0.19	0.18	0.16	0.14	0.16	0.14	0.15
45	0.31	0.31	0.30	0.32	0.31	0.23	0.22	0.24	0.23	0.22	0.18	0.17	0.18	0.16	0.17
60	0.34	0.33	0.32	0.34	0.33	0.26	0.27	0.28	0.27	0.28	0.22	0.20	0.20	0.18	0.19
90	0.35	0.35	0.34	0.36	0.35	0.30	0.31	0.31	0.30	0.32	0.24	0.22	0.22	0.21	0.20
120	0.38	0.37	0.36	0.38	0.37	0.33	0.32	0.33	0.33	0.34	0.25	0.24	0.24	0.23	0.24

*Concentration based on active ingredient (50 per cent of commercial preparation).

Conditions:

1. Each Warburg flask contained:
   1 ml 0.3M succinic acid
   1 ml tissue homogenate
   1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-4 (Continued)

Oxygen Uptake by Trout Tissue Homogenates
Exposed to Pesticides

Malathion*

Time Minutes	Oxygen Uptake, mg oxygen/g tissue														
	Liver, 22.7 g/l					Heart, 11.0 g/l					Gill, 6.7 g/l				
	Concentration, mg pesticide/mg tissue														
	0.0	0.01	0.27	0.54	2.17	0.0	0.02	0.54	1.08	4.33	0.0	0.03	0.90	1.80	7.20
5	0.17	0.16	0.17	0.15	0.16	0.11	0.10	0.09	0.10	0.10	0.05	0.04	0.05	0.05	0.05
10	0.23	0.22	0.23	0.21	0.22	0.14	0.14	0.13	0.13	0.12	0.09	0.08	0.09	0.08	0.08
20	0.29	0.29	0.28	0.28	0.27	0.16	0.15	0.14	0.15	0.15	0.11	0.10	0.10	0.11	0.10
30	0.31	0.30	0.30	0.29	0.31	0.20	0.20	0.19	0.19	0.18	0.16	0.16	0.15	0.15	0.14
45	0.32	0.31	0.31	0.31	0.30	0.24	0.23	0.23	0.22	0.23	0.19	0.18	0.17	0.18	0.19
60	0.33	0.33	0.32	0.32	0.32	0.26	0.26	0.24	0.25	0.26	0.23	0.22	0.23	0.23	0.23
90	0.34	0.33	0.33	0.32	0.33	0.27	0.27	0.26	0.26	0.25	0.24	0.23	0.22	0.23	0.24
120	0.34	0.34	0.33	0.33	0.33	0.30	0.29	0.29	0.28	0.29	0.25	0.24	0.24	0.23	0.24

*Concentration based on active ingredient (57 per cent of commercial preparation).

Conditions:

1. Each Warburg flask contained:
   1 ml 0.3M succinic acid
   1 ml tissue homogenate
   1 ml surfactant solution of organics
2. All values are average of 4 runs, 8 flasks.

Table B-5

60 Minute Oxygen Uptake Values for Trout Tissue Homogenates
Exposed to Treated Missouri River Water CCE

Tissue Homogenate	Extract Concentration mg organics/mg tissue	60 Minute Oxygen Uptake % Control
Liver	0.015	100
	0.03	97
	0.045	91
	0.06	74
	0.09	54
	0.24	23
	0.365	6
Heart	0.03	100
	0.06	97
	0.09	93
	0.12	80
	0.18	57
	0.48	20
	0.73	7
Gill	0.05	96
	0.10	96
	0.15	96
	0.20	83
	0.30	54
	0.80	33
	1.35	4

APPENDIX C

OXYGEN TRANSFER STUDIES

Oxygen transfer studies were undertaken in order to evaluate the mode of action of the trace organics. After an evaluation of silicone and cellulose type membranes (Table C-1), the silicone membrane was selected for these studies. The results of studies to determine the effect of different concentrations of subsurface water organic micropollutants on oxygen transfer efficiency are tabulated in Table C-2; data from studies to evaluate the cumulative effect of the subsurface water organics are summarized in Table C-3; and the results of a study using a surface water CCE are shown in Table C-4.

Table C-1

Oxygen Transfer Efficiencies of Silicone
And Cellulose Membranes

Time Minutes	Silicone Membrane			Cellulose Membrane		
	Dissolved Oxygen mg/l	Dissolved Oxygen Removed mg/l	Transfer Efficiency %	Dissolved Oxygen mg/l	Dissolved Oxygen Removed mg/l	Transfer Efficiency %
0	7.1	--	--	8.1	--	--
15	5.5	1.6	23	7.5	0.6	7
30	4.6	2.5	35	6.8	1.3	16
45	0.8	6.3	89	7.1	2.0	25
60	0.6	6.5	92	5.3	2.8	34
100	0.2	6.9	97	4.5	3.6	44
120	0.1	7.0	99	4.1	4.0	49
150	0.1	7.0	99	3.9	4.2	52
180	0.1	7.0	99	3.8	4.3	53

Table C-2

Oxygen Transfer Efficiency of Silicone Membrane
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #1 Unit #1

Time Minutes	Dissolved Oxygen mg/l			Dissolved Oxygen Removed, mg/l			Transfer Efficiency %		
	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After
	CCE, 90 mg/l								
0	9.0	7.7	8.0	--	--	--	--	--	--
5	8.6	7.4	7.6	0.4	0.3	0.4	4	4	5
15	6.9	5.8	5.7	2.1	1.9	2.3	23	25	29
30	4.9	3.6	4.0	4.1	4.1	4.0	45	53	50
45	4.0	2.8	3.0	5.0	4.9	5.0	55	67	62
60	2.8	1.8	2.0	6.2	5.9	6.0	69	77	75
100	1.8	1.0	1.0	7.2	6.7	7.0	80	87	88
120	1.0	0.8	0.8	7.9	6.9	7.2	87	90	90
150	0.8	0.6	0.6	8.2	7.1	7.4	91	92	92
180	0.6	0.2	0.6	8.4	7.1	7.4	93	92	92
	CAE, 220 mg/l								
0	8.0	10.3	8.3	--	--	--	--	--	--
5	7.6	9.6	7.7	0.4	0.4	0.6	5	4	7
15	5.7	7.4	5.6	2.3	2.9	2.7	29	28	32
30	4.0	5.3	4.0	4.0	5.0	4.3	50	48	52
45	3.0	4.3	3.1	5.0	6.0	5.2	62	58	63
60	2.0	3.5	2.3	6.0	6.8	6.0	75	66	72
100	1.0	2.4	1.2	7.0	7.9	7.1	88	77	86
120	0.8	1.2	0.9	7.2	9.1	7.4	90	88	89
150	0.6	0.9	0.8	7.4	9.4	7.5	92	91	90
180	0.6	0.8	0.8	7.4	9.5	7.5	92	92	90
	CCE & CAE, 138 mg/l								
0	8.3	11.4	7.2	--	--	--	--	--	--
5	7.7	11.0	7.1	0.6	0.4	0.1	7	3	1
15	5.6	10.3	7.0	2.7	1.1	0.2	33	10	3
30	4.0	10.0	6.7	4.3	1.4	0.5	52	12	7
45	3.1	9.7	6.6	5.2	1.7	0.6	63	15	8
60	2.3	9.1	6.4	6.0	2.3	0.8	72	20	11
100	1.2	8.2	6.3	7.1	3.2	0.9	86	28	12
120	0.9	7.7	6.2	7.4	3.7	1.0	89	32	14
150	0.8	7.2	6.0	7.5	4.2	1.2	91	37	17
180	0.8	7.2	6.0	7.5	4.2	1.2	91	37	17

Table C-2 (Continued)

Oxygen Transfer Efficiency of Silicone Membrane
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #2 Unit #1

Time Minutes	Dissolved Oxygen mg/l			Dissolved Oxygen Removed, mg/l			Transfer Efficiency %		
	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After
	CCE, 100 mg/l								
0	8.0	10.0	7.7	--	--	--	--	--	--
5	7.4	9.4	6.8	0.6	0.4	0.6	8	4	8
15	5.6	7.4	5.1	2.4	2.6	2.3	30	26	31
30	3.1	5.0	2.5	4.9	5.0	4.9	60	50	66
45	2.0	4.1	1.5	6.0	5.9	5.9	75	59	80
60	1.1	3.0	0.8	6.9	7.0	6.6	86	70	89
100	0.5	2.5	0.4	7.5	7.5	7.0	94	75	95
120	0.3	2.6	0.3	7.7	8.0	7.1	96	80	96
150	0.3	1.5	0.3	7.7	8.5	7.1	96	85	96
180	0.3	1.0	0.3	7.7	9.0	7.1	96	90	96
	CAE, 220 mg/l								
0	9.0	10.0	8.6	--	--	--	--	--	--
5	8.4	9.5	8.0	0.6	0.5	0.6	7	5	7
15	6.3	7.5	5.9	2.7	2.5	2.7	30	25	37
30	3.5	5.0	3.3	5.5	5.0	5.3	60	50	62
45	2.1	3.5	2.0	6.9	6.5	6.6	77	65	77
60	1.4	2.6	1.5	7.6	7.4	7.1	84	74	82
100	0.7	1.9	0.9	8.3	8.1	7.7	92	81	90
120	0.4	1.1	0.6	8.6	8.9	8.0	94	89	93
150	0.3	0.9	0.4	8.7	9.1	8.2	95	91	95
180	0.3	0.8	0.3	8.7	9.2	8.3	95	92	96
	CCE & CAE, 260 mg/l								
0	7.7	10.0	9.3	--	--	--	--	--	--
5	7.1	9.7	9.2	0.6	0.3	0.1	8	3	1
15	5.5	9.3	8.9	2.2	0.7	0.4	29	7	4
30	2.6	9.1	8.7	5.1	0.9	0.6	66	9	6
45	1.2	8.8	8.4	6.5	1.2	0.9	84	12	10
60	0.6	8.5	8.1	7.1	1.5	1.2	92	15	13
100	0.2	7.5	7.5	7.5	2.5	1.8	97	25	19
120	0.2	7.3	7.3	7.5	2.7	2.0	97	27	21
150	0.2	7.2	7.1	7.5	2.8	2.2	97	28	24
180	0.2	7.2	7.0	7.5	2.8	2.3	97	28	25

Table C-2 (Continued)

Oxygen Transfer Efficiency of Silicone Membrane
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #2 Unit #1

Time Minutes	Dissolved Oxygen mg/l			Dissolved Oxygen Removed, mg/l			Transfer Efficiency %		
	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After
	CCE & CAE, 65 mg/l								
0	10.5	8.5	7.7	--	--	--	--	--	--
5	9.2	8.2	7.3	1.3	0.3	0.4	12	4	6
15	8.4	7.9	7.1	2.1	0.6	0.6	20	7	8
30	7.1	7.5	6.9	3.4	1.0	0.8	32	12	11
45	5.2	7.0	6.6	5.3	1.5	1.1	50	18	14
60	4.2	6.5	6.5	6.3	2.0	1.2	60	23	16
100	2.6	5.6	5.8	7.9	2.9	1.9	75	34	25
120	1.5	5.4	5.4	9.0	3.1	2.3	86	37	30
150	1.3	5.2	5.3	9.2	3.3	2.4	88	39	32
180	1.0	5.1	5.2	9.5	3.4	2.5	90	40	33
	CCE & CAE, 130 mg/l								
0	9.4	7.2	9.2	--	--	--	--	--	--
5	8.9	7.1	9.0	0.5	0.1	0.2	6	1	3
15	7.1	7.0	8.8	2.3	0.2	0.4	25	3	4
30	5.6	6.7	8.6	3.8	0.5	0.6	40	7	5
45	4.5	6.3	8.5	4.9	0.9	0.7	52	13	10
60	3.7	5.6	8.4	5.7	1.6	0.8	61	23	16
100	2.7	5.5	8.0	6.7	1.7	1.2	72	24	17
120	2.0	5.2	7.9	7.4	2.0	1.3	79	28	21
150	1.3	5.0	7.9	8.1	2.2	1.3	86	30	25
180	1.2	5.0	7.9	8.2	2.2	1.3	87	30	27

Table C-2 (Continued)

Oxygen Transfer Efficiency of Silicone Membrane
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #2 Unit #2

Time Minutes	Dissolved Oxygen mg/l			Dissolved Oxygen Removed, mg/l			Transfer Efficiency %		
	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After
	CCE, 110 mg/l								
0	7.6	8.5	6.0	--	--	--	--	--	--
5	7.2	8.6	5.5	0.4	0.5	0.5	5	6	8
15	6.0	6.0	3.5	1.6	2.0	2.5	21	24	41
30	4.8	4.8	2.8	2.8	3.7	3.2	37	44	53
45	4.0	4.0	2.4	3.6	4.5	3.6	47	53	60
60	3.0	3.5	2.0	4.6	5.0	4.0	60	59	67
100	1.9	2.5	1.0	5.7	6.0	5.0	75	71	83
120	1.0	1.0	0.6	6.6	7.5	5.4	87	88	90
150	0.6	0.8	0.2	7.0	7.7	5.8	92	91	97
180	0.5	0.7	0.2	7.1	7.8	5.8	93	92	97
	CAE, 260 mg/l								
0	6.0	6.3	6.2	--	--	--	--	--	--
5	5.5	6.0	5.9	0.5	0.3	0.3	8	5	5
15	3.5	4.9	4.2	2.5	1.4	2.0	41	22	32
30	2.8	4.0	3.1	3.2	2.3	3.1	53	37	50
45	2.4	3.0	2.4	3.6	3.3	3.8	60	52	61
60	2.0	2.2	1.4	4.0	4.1	4.8	67	65	77
100	1.0	1.4	0.9	5.0	4.9	5.3	83	78	85
120	0.6	0.8	0.6	5.4	5.5	5.6	90	87	90
150	0.2	0.4	0.6	5.8	5.9	5.6	97	94	90
180	0.2	0.4	0.6	5.8	5.9	5.6	97	94	90
	CCE & CAE, 176 mg/l								
0	9.2	8.8	6.6	--	--	--	--	--	--
5	7.8	8.6	6.5	1.4	0.2	0.1	15	2	2
15	5.8	8.4	6.4	3.4	0.4	0.2	37	4	3
30	2.7	8.3	6.3	6.5	0.5	0.3	71	6	5
45	1.2	8.2	6.2	8.0	0.6	0.4	87	7	6
60	0.9	8.0	6.1	8.3	0.8	0.5	90	9	8
100	0.7	7.8	5.8	8.5	1.0	0.8	92	12	11
120	0.4	7.7	5.5	8.8	1.1	1.1	96	17	12
150	0.2	7.5	5.4	9.0	1.3	1.2	98	18	15
180	0.2	7.4	5.4	9.0	1.4	1.2	98	18	16

Table C-2 (Continued)

Oxygen Transfer Efficiency of Silicone Membrane
Exposed to Subsurface Water CCE and CAE

Meramec Spring Run #2 Unit #3

Time Minutes	Dissolved Oxygen mg/l			Dissolved Oxygen Removed, mg/l			Transfer Efficiency %		
	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After
	CCE, 100 mg/l								
0	7.3	6.3	8.0	--	--	--	--	--	--
5	6.6	5.8	7.6	0.7	0.5	0.4	10	8	5
15	4.8	4.0	6.2	2.5	2.3	1.8	34	37	22
30	3.7	3.4	5.2	3.6	2.9	2.8	49	46	35
45	3.2	3.0	4.7	4.1	3.3	3.3	56	52	41
60	2.5	2.2	3.4	4.8	4.1	4.6	66	65	58
100	1.8	1.7	2.6	5.5	4.6	5.4	75	73	68
120	1.3	1.0	1.3	6.0	5.3	6.7	82	84	84
150	0.7	0.6	0.7	6.6	5.7	7.3	91	90	91
180	0.7	0.6	0.7	6.6	5.7	7.3	91	90	91
	CAE, 220 mg/l								
0	8.0	8.8	6.1	--	--	--	--	--	--
5	7.6	8.2	5.5	0.4	0.6	0.6	5	7	10
15	6.2	6.3	4.8	1.8	2.5	1.3	22	28	21
30	5.2	5.5	3.6	2.8	3.3	2.5	35	37	41
45	4.7	4.9	3.0	3.3	3.9	3.1	41	44	51
60	3.4	3.6	2.6	4.6	5.2	3.5	58	59	57
100	2.6	2.7	2.0	5.4	6.1	4.1	68	69	67
120	1.3	1.0	1.4	6.7	7.8	4.7	84	89	77
150	0.7	0.6	0.8	7.3	8.2	5.3	91	93	87
180	0.7	0.6	0.8	7.3	8.2	5.3	91	93	87
	CCE & CAE, 190 mg/l								
0	6.1	8.2	8.3	--	--	--	--	--	--
5	5.5	7.6	7.5	0.6	0.6	0.8	10	7	10
15	4.8	6.6	4.9	1.3	1.6	3.4	21	20	41
30	3.6	5.0	3.8	2.5	3.2	4.5	41	39	54
45	3.0	4.0	3.3	3.1	4.2	5.0	51	51	60
60	2.6	3.5	2.6	3.5	4.7	5.7	57	57	69
100	1.9	2.0	2.0	4.2	6.2	6.3	69	76	76
120	1.2	1.1	1.4	4.9	7.1	6.9	80	86	83
150	0.6	0.9	0.8	5.5	7.3	7.5	90	89	90
180	0.6	0.9	0.8	5.5	7.3	7.5	90	89	90

Table C-3

Cumulative Effect of Meramec Spring Run #2 Unit #1 CCE and CAE
On Oxygen Transfer Efficiency of Silicone Membrane

Time Minutes	Dissolved Oxygen mg/l			Dissolved Oxygen Removed, mg/l			Transfer Efficiency %		
	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After
	CCE & CAE, 13 mg/l								
0	7.2	7.5	9.0	--	--	--	--	--	--
5	6.6	7.2	8.5	0.6	0.3	0.5	8	4	6
15	5.4	6.1	7.3	1.8	1.4	1.7	25	19	20
30	4.6	5.3	6.3	2.6	2.2	2.7	36	29	30
45	3.7	4.3	5.2	3.5	3.2	3.8	49	43	42
60	2.9	3.5	4.0	4.3	4.0	5.0	60	53	55
100	1.9	3.3	3.2	5.3	4.2	5.8	74	56	64
120	1.6	1.8	2.6	5.6	5.7	6.4	68	63	71
150	1.4	1.4	2.1	6.1	5.8	6.9	81	80	76
180	1.2	1.2	2.0	6.3	6.0	7.0	84	83	77
	CCE & CAE, 40 mg/l								
0	9.0	8.6	10.0	--	--	--	--	--	--
5	8.5	8.1	9.4	0.5	0.5	0.6	6	6	6
15	7.3	7.0	8.0	1.7	1.6	2.0	20	19	20
30	6.3	6.0	7.1	2.7	2.6	2.9	30	30	29
45	5.2	5.0	6.5	3.8	3.6	3.5	42	42	35
60	4.0	4.0	6.1	5.0	4.6	3.9	55	53	39
100	3.2	3.6	5.6	5.8	5.0	4.4	64	58	44
120	2.6	3.3	5.0	6.4	5.3	5.0	71	62	50
150	2.1	3.1	4.1	6.9	5.5	5.9	76	64	59
180	2.0	3.0	4.0	7.0	5.6	6.0	77	65	60
	CCE & CAE, 65 mg/l								
0	10.0	8.7	7.5	--	--	--	--	--	--
5	9.4	8.2	7.3	0.6	0.5	0.2	6	6	3
15	8.0	8.1	7.1	2.0	0.6	0.4	20	7	5
30	7.1	8.0	7.0	2.9	0.7	0.5	29	8	6
45	6.5	7.6	6.7	3.5	1.1	0.8	35	13	11
60	6.1	7.1	6.4	3.9	1.6	1.1	39	18	15
100	5.6	6.8	6.2	4.4	1.9	1.3	44	22	18
120	5.0	6.5	6.0	5.0	2.2	1.5	50	25	20
150	4.1	6.4	5.7	5.9	2.3	1.8	59	27	24
180	4.0	6.3	5.6	6.0	2.4	1.9	60	28	25

Table C-4

Oxygen Transfer Efficiency of Silicone Membrane
Exposed to Treated Missouri River Water CCE

Time Minutes	Dissolved Oxygen mg/l			Dissolved Oxygen Removed, mg/l			Transfer Efficiency %		
	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After	Tap H_2O Before	Extract	Tap H_2O After
	CCE, 76 mg/l								
0	7.1	9.7	8.0	--	--	--	--	--	--
5	6.6	9.2	7.4	0.5	0.5	0.6	7	5	8
15	4.6	7.3	5.8	2.5	2.4	2.2	35	25	27
30	2.4	5.0	3.3	4.5	4.7	4.7	65	49	59
45	1.5	4.0	2.3	5.6	5.7	5.7	78	59	71
60	0.9	3.0	1.9	6.2	6.7	6.1	86	70	76
100	0.7	2.6	1.5	6.4	7.1	6.5	89	74	81
120	0.5	2.4	0.8	6.6	7.3	7.2	92	76	90
150	0.3	2.0	0.6	6.8	7.7	7.4	94	80	93
180	0.3	2.0	0.6	6.8	7.7	7.4	94	80	93

APPENDIX D

PREDICTION OF LONG-TERM TOXICITY LEVELS

Because of the length of time required to evaluate the long-term (eventual) toxic effects, a method for predicting long-term toxic levels from short-term data was developed. This method employed a toxicity equation defined (see Equation 3, p. 85) as follows:

$$y_t = y_c + (y_o - y_c)\, e^{-bt}$$

where: y_t = toxicity factor at time t
y_c = toxicity factor corresponding to the long-term TLm value
y_o = toxicity factor corresponding to immediate TLm value
b = a constant depending on the test material and test fish

The toxicity factor related the toxicant concentration and physical characteristics of the test fish, and its form depended on the mode of action of the toxicant. The factor was defined for subsurface water organics (see Equation 1, p. 84) as

$$y_t = C_t \cdot \left[\frac{GSA}{K}\right]^{1.25}$$

where: C_t = TLm value at time t, mg/l
GSA = Gill surface area, mm^2
K = condition factor, weight/(length)^3, g/cm^3

because these materials affected the physical transfer of oxygen across the gills; while for surface water organics and pesticides, it was defined (see Equation 6, p. 90) as

$$y_t = C_t/K$$

because these materials did not affect the physical transfer of oxygen across the gills.

In order to apply the general toxicity equation to a given combination of toxicant and test fish, it was necessary to evaluate the numerical values of the constants y_o, y_c, and b on the basis of acute toxicity data. The value of y_o was determined by plotting the toxicity factor against time on a semilogarithmic scale, fitting a straight line to the various points, extending the line to the y_t axis, and determining the intercept. This value could also be approximated experimentally using the toxicant concentration which killed one-half of the test fish within a very short period of time (usually 2 hours). The value of the constants y_c and b was then determined by simultaneous trial and error solution of the toxicity equation at two times, t_1 and t_2. A relationship between the corresponding equations at times t_1 and t_2 was derived as follows:

$$y_{t_1} = y_c + (y_o - y_c)\, e^{-bt_1} \qquad y_{t_2} = y_c + (y_o - y_c)\, e^{-bt_2}$$

and

$$e^{-bt_1} = \frac{y_{t_1} - y_c}{y_o - y_c} \qquad e^{-bt_2} = \frac{y_{t_2} - y_c}{y_o - y_c}$$

raising both sides to the power t_2 or t_1, as appropriate

$$\left[e^{-bt_1}\right]^{t_2} = \left[\frac{y_{t_1} - y_c}{y_o - y_c}\right]^{t_2} \qquad \left[e^{-bt_2}\right]^{t_1} = \left[\frac{y_{t_2} - y_c}{y_o - y_c}\right]^{t_1}$$

and since

$$\left[e^{-bt_1}\right]^{t_2} = e^{-bt_1 t_2} = \left[e^{-bt_2}\right]^{t_1}$$

it follows that

$$\frac{\left[\bar{y}_{t_1} - y_c\right]^{t_2}}{\left[y_o - y_c\right]} = \frac{\left[\bar{y}_{t_2} - y_c\right]^{t_1}}{\left[y_o - y_c\right]}$$

or

$$\frac{\left[\bar{y}_{t_1} - y_c\right]^{t_2}}{\left[y_o - y_c\right]^{t_2 - t_1}} = \left[y_{t_2} - y_c\right]^{t_1} \qquad \text{(Equation D-1)}$$

For additional ease in computations, t_1 could be chosen as one day and the relationship simplified to

$$\frac{\left[\bar{y}_{t_1} - y_c\right]^{t_2}}{\left[y_o - y_c\right]^{t_2 - 1}} + y_c = y_{t_2} \qquad \text{(Equation D-2)}$$

which was then solved by trial and error.

The toxicity equation constants for the organic micropollutants and pesticides employed in this study were determined using the above outlined procedure and are summarized in Table D-1. To illustrate numerically the various steps undertaken, the calculations pertaining to the development of the toxicity equation for Meramec Spring Run #2 Unit #2 CCE and CAE materials and trout are presented in the following pages.

The following experimental toxicity factors were available from the acute data (see Table 17, p. 86):

Table D-1

Toxicity Equation Constants for Organic Micropollutants and Pesticides

Test Material	Test Fish	y_o		y_c				b
		Experimental	Extrapolated	Evaluated at t_1 and t_2, days			Average	
				1 and 5	1 and 4	2 and 5		
Meramec Spring Run #2 Unit #1 CCE & CAE	Trout	4.0×10^7	3.8×10^7	0.86×10^7	0.83×10^7	0.86×10^7	0.85×10^7	0.17
	Sunfish	12.8×10^7	13.4×10^7	1.81×10^7	1.80×10^7	1.78×10^7	1.80×10^7	0.15
Unit #2 CCE & CAE	Trout	2.6×10^7	2.8×10^7	0.10×10^7	0.11×10^7	0.10×10^7	0.10×10^7	0.11
	Sunfish	13.0×10^7	13.2×10^7	4.20×10^7	4.22×10^7	4.18×10^7	4.20×10^7	0.13
	Red Shiners	88.0×10^7	86.0×10^7	4.00×10^7	4.10×10^7	3.89×10^7	4.00×10^7	0.11
	Golden Shiners	61.0×10^7	65.5×10^7	47.6×10^7	47.4×10^7	47.4×10^7	47.5×10^7	0.32
Missouri River at St. Louis CCE	Trout	23.0×10^2	21.0×10^2	0.29×10^2	0.30×10^2	0.30×10^2	0.30×10^2	0.11
	Sunfish	26.0×10^2	25.0×10^2	11.9×10^2	11.9×10^2	11.6×10^2	11.8×10^2	0.27
	Golden Shiners	45.5×10^2	46.0×10^2	5.0×10^2	4.80×10^2	5.19×10^2	5.0×10^2	0.18
Malathion	Trout	4.7×10^{-1}	4.67×10^{-1}	0.11×10^{-2}	0.1×10^{-2}	0.1×10^{-2}	0.1×10^{-2}	0.23
	Red Shiners	29.0×10^{-1}	26.7×10^{-1}	1.88×10^{-1}	1.9×10^{-1}	1.93×10^{-1}	1.9×10^{-1}	0.15
Sevin	Trout	2.3×10^2	2.2×10^2	2.0×10^1	2.0×10^1	1.96×10^1	2.0×10^1	0.44
	Red Shiners	12.0×10^2	10.5×10^2	1.3×10^2	1.5×10^2	1.41×10^2	1.4×10^2	0.18

time, days:		toxicity factor:	
	1		$y_1 = 2.48 \times 10^7$
	2		$y_2 = 2.24 \times 10^7$
	4		$y_4 = 1.79 \times 10^7$
	5		$y_5 = 1.56 \times 10^7$

These values were plotted on semilogarithmic paper (Figure D-1) and by extrapolation

$$y_o = 2.8 \times 10^7$$

was determined. The value of the constant y_c was computed by solving Equation D-2 for 1 and 5 days as follows:

$$\frac{\left[y_{t_1} - y_c\right]^{t_2}}{\left[y_o - y_c\right]^{t_2 - 1}} + y_c = y_{t_2} \qquad \text{(Equation D-2)}$$

substituting the corresponding values

$$\frac{\left[2.48 \times 10^7 - y_c\right]^5}{\left[2.80 \times 10^7 - y_c\right]^4} + y_c = 1.56 \times 10^7$$

assuming $y_c = 0.20 \times 10^7$

$$\frac{\left[2.28 \times 10^7\right]^5}{\left[2.6 \times 10^7\right]^4} + 0.2 \times 10^7 = 1.56 \times 10^7$$

$$1.40 \times 10^7 + 0.2 \times 10^7 = 1.56 \times 10^7$$

$$1.60 \times 10^7 = 1.56 \times 10^7$$

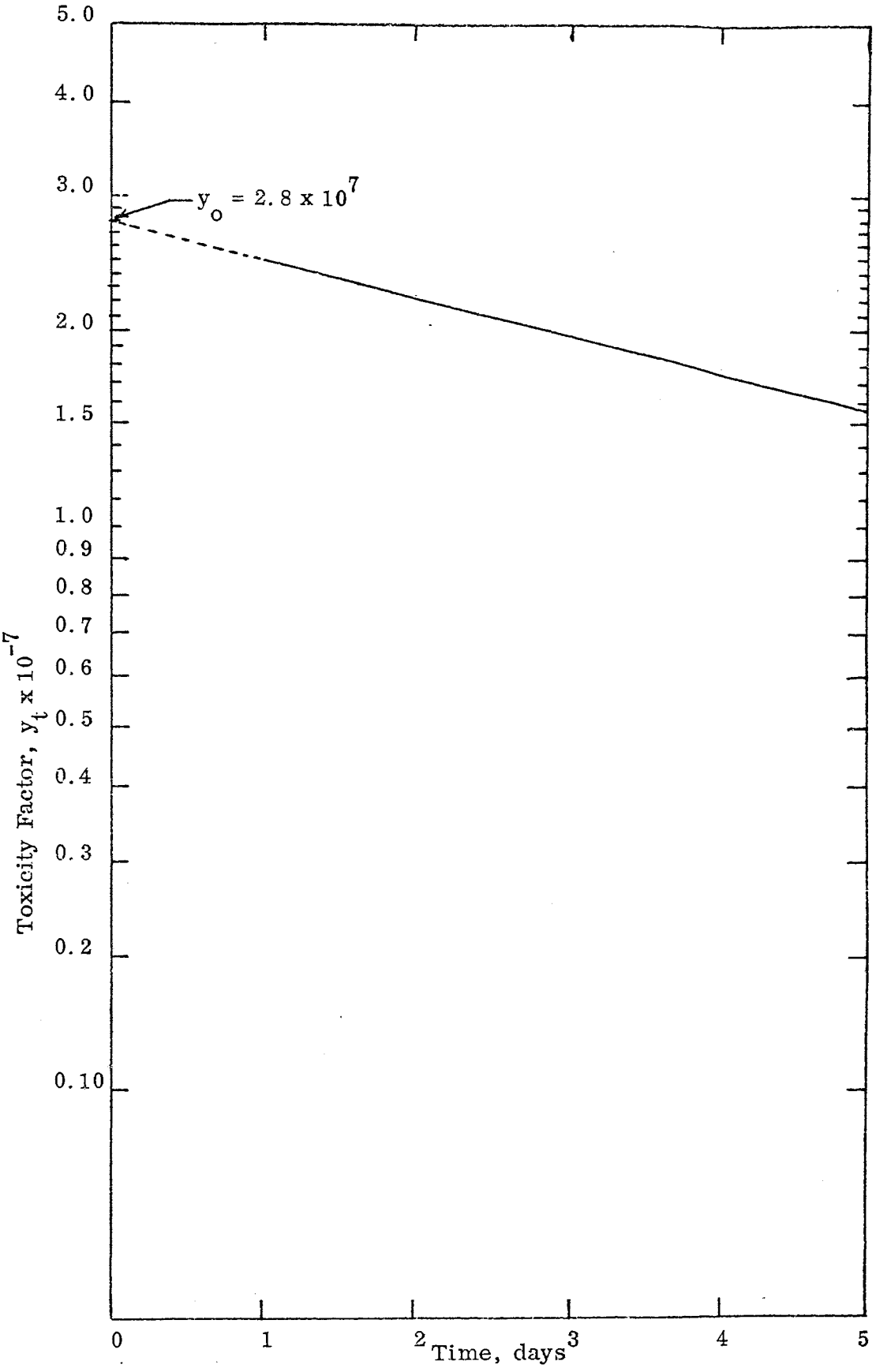

Figure D-1

Determination of Constant y_o For
Meramec Spring Run #2 Unit #2 CCE and CAE and Trout

assuming $y_c = 0.10 \times 10^7$

$$\frac{\left[2.38 \times 10^7\right]^5}{\left[2.7 \times 10^7\right]^4} + 0.1 \times 10^7 = 1.56 \times 10^7$$

$$1.46 \times 10^7 + 0.1 \times 10^7 = 1.56 \times 10^7$$

$$1.56 \times 10^7 = 1.56 \times 10^7$$

Therefore, a value of

$$y_c = 0.10 \times 10^7$$

was accepted. Common four place logarithms were employed in the calculations. The toxicity equation for this extract and test fish can now be written as

$$y_t = 0.10 \times 10^7 + (2.7 \times 10^7)\, e^{-bt}$$

The value of the constant b was then evaluated using this equation at two different times, 1 and 4 days:

$$y_1 = 2.48 \times 10^7 = 0.10 \times 10^7 + (2.7 \times 10^7)\, e^{-b}$$

$$e^{-b} = \frac{2.38 \times 10^7}{2.7 \times 10^7}$$

$$e^{-b} = 0.882$$

$$b = 0.11$$

and

$$y_4 = 1.79 \times 10^7 = 0.10 \times 10^7 + (2.7 \times 10^7)\, e^{-4b}$$

$$e^{-4b} = \frac{1.69 \times 10^7}{2.7 \times 10^7}$$

$$e^{-4b} = 0.627$$

$$4b = 0.455$$

$$b = 0.113$$

and the constant was taken as:

$$b = 0.11$$

Finally, the toxicity equation for Meramec Spring Run #2 Unit #2 CCE and CAE materials and trout can be written as:

$$y_t = 0.1 \times 10^7 + (2.7 \times 10^7)\, e^{-0.11t}$$

VITA

John W. Smith was born on November 18, 1943, in De Soto, Missouri, where he received his elementary and secondary education. He entered the University of Missouri School of Mines and Metallurgy, Rolla, Missouri, in the summer of 1961 and received the degree of Bachelor of Science in Civil Engineering from the University of Missouri at Rolla (formerly the University of Missouri School of Mines and Metallurgy) in June 1965. Following graduation, the author entered graduate school at the University and received the degree of Master of Science in Civil Engineering in June 1967.

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He is the author of the following publications:

"The Recovery and Characterization of Organic Micropollutants from Missouri Subsurface Waters," M. S. Thesis, University of Missouri at Rolla, 1967.

"Trace Organics in Missouri Subsurface Waters," (coauthor) Journal American Water Works Association, 60, 586 (1968).

He was married to Miss Karen Fusselman on December 19, 1964.